CHAPTER 1
INTRODUCTION

*E. coli*, was first described by *Theodor Escherich* in 1885, is a member of family enteriobacteriaeace. It is a gram negative, motile, non-sporing bacillus, produces rose pink colonies on MacConkey Agar. The species can be differentiated from other members of enterobacteria by biochemical reaction, being a member of enterobacteriaeace it is present as normal flora in the lower intestine of both humans and animals; However, some strains can cause gastrointestinal illness ranging from mild to cholera-like diarrhoea and may lead to potentially fatal complications, such as haemolytic uremic syndrome (HUS) & thrombotic thrombocytopenic purpura in human beings (Hussein *et al.*, 2007; Ramamurthy *et.al*, 2008). *E. coli* is a genetically and phenotypically diverse species whose strains are identified on the basis of ‘O’, ‘H’ and sometimes ‘K’ antigens, biochemically & serologically based on toxigenicity, On the basis of intestinal diseases, there are six categories: enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enteroinvasive *E. Coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and enterotoxigenic *E. coli* (ETEC) (Nataro *et.al*, 1998). Over 700 serotypes of *E. coli* are recognized on the basis of O, H, and K antigens.

Several highly adapted clones have acquired specific virulence factors that are accountable for a variety of intestinal and extraintestinal diseases including diarrhea, acute inflammation, hemorrhagic colitis, urinary tract infections, septicemia, and neonatal meningitis (Kaper *et al.*, 2004; Levine *et al.*, 1987; Nataro *et al.*, 1998; Orskov *et al.* 1992). In rare cases, virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and Gram-negative pneumonia. Certain
strains of *E. coli*, such as O157:H7, O104:H4, O121, O26, O103, O111, O145, and O104:H21, produce potentially lethal toxins. Principally, Shiga like toxin producing *E. coli* (STEC) is transmitted through the consumption of contaminated foods such as raw or undercooked ground meat products and raw milk (Clark *et al*., 1994). This particular strain is linked to the 2006 United States *E. coli* outbreak due to fresh spinach. The O104:H4 strain is equally virulent. Antibiotic and supportive treatment protocols for it are not as well-developed (it has the ability to be enterohemorrhagic like O157:H7, causing bloody diarrhea, but also is more enteroaggregative, meaning it adheres well and clumps to intestinal membranes). It is the strain behind the ongoing and deadly June 2011 *E. coli* outbreak in Europe (Scheutz *et al*., 2011)

If *E. coli* bacteria escape the intestinal tract through a perforation (for example from an ulcer, a ruptured appendix, or due to a surgical error) and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. However, *E. coli* are extremely sensitive to such antibiotics as streptomycin or gentamicin. Recent research suggests treatment of enteropathogenic *E. coli* with antibiotics may not improve the outcome of the disease, as it may significantly increase the chance of developing haemolytic-uremic syndrome.

The diseases caused by a particular strain of *E. coli* depend upon distribution and expression of an array of virulence determinants, including adhesins, invasins, toxins, and abilities to withstand host defenses. Virulence Determinants of Pathogenic *E. coli* are:

- **Adhesins**: CFAI/CFAII, Type 1 fimbriae, P fimbriae, S fimbriae, Intimin (non-fimbrial adhesin), EPEC adherence factor, Invasins, hemolysin, Shigella-like "invasins" for intracellular invasion and spread, Motility/chemotaxis, flagella, Toxins - LT toxin, ST toxin, Shiga toxin, cytotoxins, endotoxin, Antiphagocytic surface properties-

Cattle are known to be the most important reservoirs of pathogenic *E. coli* strains across the world. The pathogen is known to be shed in manure by cattle and survives in animal waste and wastewater (Avery *et al.*, 2005; Elder *et al.*, 2000; Hancock *et al.*, 1994; Ibekwe *et al.*, 2002; Laegried *et al.*, 1999). Other animals known to harbour pathogenic *E. coli* strains, with or without symptoms, include sheep, goats (Beutin *et al.*, 1993), pigs (Mohammad *et al.*, 1985), buffaloes, horses (Peeters *et al.*, 1994), deer (Knee *et al.*, 1997), wild boar, reindeer (Pierard *et al.*, 1994), birds, etc. Cattle hides have been identified as an important source of microbial contamination of carcasses (Ridell and Korkeala, *et al.*, 1993; Bell, *et al.*, 1997). It has been shown that O157:H7 and non-O157:H7 STEC can be easily transferred from cattle hides to the carcass (Barkocy-Gallagher, *et al.*, 2003). Pathogenic *E. coli* can potentially enter the human food chain most commonly through contamination with faeces or intestinal contents after slaughter. The organism may also be transmitted from person to person through the faeco–oral route.
Pathogenic *E. coli* once ingested by a man, it moves through the digestive tract and settles in the intestine and can lead to illness. Normally, It is inhabitant of intestinal micro flora of dairy herds and can be linked to unpasteurized milk (Martin, *et al.*, 1986; Herriott, *et al.*, 1994; Lahti, *et al.*, 2002), spinach and various vegetables (Cieslak, *et al.*, 1993), because of their close contact with dairy herd feces as the latter is used as manure in the fields of vegetables, sometimes untreated wastewater also used for irrigation purpose which also increases the probability of contamination. Pasteurized milk treated
manure & wastewater will kill the bacteria & prevent the food borne or waterborne infections.

It continues to be the challenge for the people residing in the rural areas where the peoples live in close proximity of cattle & have least knowledge about the pathogenicity of the bacteria. As it continually adapts to different conditions and environments. The organism can remain viable for months together in the soil. It can survive and replicate in both stagnating and free-flowing water. Unlike many other bacteria, pathogenic *E.coli* can survive and replicate in aerobic as well as anaerobic environments. It can respond and adapt to differences in environmental changes such as; chemicals, pH, and temperature in remarkable ways. Worldwide, virulent strains of *E. coli* also are emerging, they have the potential to cause food borne illness. Researchers are working to better understand these other strains and their potential impact on various food products.

According to above information, it is clear that pathogenesis of *E.coli* is multifactorial and involves several virulence attributes of the organism. Development of cost effective vaccines for human use would be helpful for efficient management of these infection. Before this can happen, there must be at least an effective treatment strategy that will reduce the progression of infection to HUS. The purpose of this study was to produce an efficient reporting system based on public awareness to determine the epidemiology and importance of infection caused by pathogenic *E.coli* in the area for efficient management of the problem.
1.2: AIM & OBJECTIVES OF STUDY

AIM
Isolation & molecular characterization of *Escherichia coli* O157:H7 & other pathogenic *E.coli* from Dairy herd’s feaces & wastewater of Solan District of Himachal Pradesh.

OBJECTIVES
This study was planned with following objectives :

- Collection of herd’s feaces and wastewater sample from different locations at Solan & surrounding villages also.
- Isolation of *E.coli* from collected samples.
- Biochemical characterization.
- Serotype characterization.
- Genotyping of isolates.