جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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كلية الطب البيطري

دراسة مناعية في الارانب على مستحضرين من المستضدات لبكتيريا الايشريشيا القولونيا *O157:H7*.

رسالة مقدمة إلى مجلس كلية الطب البيطري – جامعة ديالى كجزء من متطلبات نيل درجة الماجستير في

الطب الباطني والوقائي البيطري/الأمراض المشتركة

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1-Introduction

Escherichia coli is a part of the normal microflora of the gastrointestinal tract of mammals and birds, but certain strains have been associated with gastrointestinal diseases in both humans and animals. These *E. coli* strains have been categorized into pathogenicity groups, based on their virulence properties (Al-mohanna., 2011).

Vero toxin-producing/Shiga toxin-producing Escherichia *coli* (VTEC/STEC) which include its well-known subgroup enterohaemorrhagic *E. coli* (EHEC).This group is characterized by the production of potent cytotoxins that inhibit protein synthesis within eukaryotic cells. These toxins are either termed verocytotoxins (VT), or Shiga toxins (Stx). Therefore, these strains are either termed Stx-producing *E. coli* (STEC) or VT-producing *E. coli* (VTEC). (Dulo., 2014)

Shiga toxin-producing *Escherichia coli* infections have been described in a wide range of both domestic and wild animal species, but their natural pathogenic role has been demonstrated only in young calves (diarrhea or dysentery), weaning pigs (edema disease), and dogs (cutaneous and renal vasculopathy in greyhounds) (Wieler and Bauerfeind., 2003).

In human beings, conversely, STEC infections are relatively uncommon, but they can cause severe illnesses such as hemorrhagic colitis and hemolytic uremic syndrome (HUS), especially among children and the elderly (Ochoa and Cleary., 2003).

The majority of the cases of disease worldwide are caused by strains of serotype O157:H7, (Tozzi *et al*.,2003.; Márquez-Velasco *et al.,* 2007).

Vero toxin-producing *Escherichia coli* represent the only pathogenic group of *E. coli* that has a definite zoonotic origin, with cattle being recognized as the major reservoir for human infections.(Caprioli *et al*.,2005). STEC can be further classified into different serotypes depending on their O

(lipopolysaccharide) and H (flagellar) antigens. Hundreds of STEC serotypes have been identified that cause disease in humans. *E. coli* O157:H7 is the most frequently isolated serotype in North America, but there are six other prevalent serogroups responsible for foodborne illness which are found across the continent: O26, O45, O103, O111, O121, and O145 (Brooks *et al.*, 2005).

Shiga toxin-producing Escherichia coli infection occurs predominantly in children and causes watery diarrhea which may be followed by bloody stool, severe abdominal cramps, and vomiting, clinically referred to as hemorrhagic colitis (Cleary, 2004). Around 5-8% of children with hemorrhagic colitis will develop hemorrhagic uremic syndrome (HUS) a condition characterized by acute kidney failure which can lead to permanent renal impairment or even death (Cleary., 2004; Mele *et al*., 2014). Each O157:H7 human infection is estimated to cost $10, 048 per case in the United States with a total economic burden of $154-$635 million per year. (Smith *et al.*, 2014). The financial burden of non-O157 infections is lower as there is no estimation of the number of deaths, which accounts for the majority of the cost (Scharff., 2012).

People are exposed to STEC O157 through a variety of sources, including direct contact with human or animal feces and indirect contact via contaminated food, water, or soil (Sargeant and Smith., 2003). The primary route of transmission of STEC O157 is contaminated food (Rangel *et al*.,2005)

There are only two vaccines commercially available licensed by the Food and Drug Administration (FDA) in USA in cattle industry which act to minimize the shedding of the bacteria, these are :

1- Type 3 secretion protein system (T3SP) based vaccine (Econiche™, BionicheLife Sciences Inc , Canada) (Andrew A *et al*.,2004; Joyce Van Donkersgoed *et al*.,2005)

2- SRP (siderophore receptor and Porin) based vaccine (Epitopix, Epitopix LLC, USA.). (Neilands.,1995 ; Thomson *et al*.,2009).

Both the TTSP and SRP vaccines work differently to inhibit STEC O157 populations in cattle intestine. Immunization of cattle with TTSP proteins (EspA, EspB, Tir, Intimin) blocks the intimate adherence of STEC O157 to intestinal epithelial cells (Potter *et al.*, 2004), while immunization of cattle with SRP proteins inhibits STEC O157 iron uptake by depriving the cell of required nutrients (Thomson *et al.*, 2009)

Several preparations have been tested experimentally in different types of animals to reach an accepted formula of vaccine to become a promising in human protection. The vaccine composing strategy was depending on the virulence factors that by which the *E.coli O57:H7* exerts its effect on host cells such as Shiga Toxin vaccines,Type 3 Secretion System-related protein vaccines, DNA vaccines , Subunit fusion vaccines and Bacterial cell-based candidates (Bacterial ghosts (BG) , live attenuated ,Killed vaccine and polysaccharide (LPS) -based vaccines).

Aims of the Study

1-Preparation of two antigens from *E.coli O157:H7* which are formaldehyde killed whole bacteria and purified bacterial Lipopolysaccharide and use these antigenic preparations as vaccine candidates.

2- Use of these two types of vaccine preparations of *E.coli O157:H7* separately to study the immune response in rabbits against these bacterial antigens via oral route and as a single dose vaccination with booster dose 15 days after primary immunization.

3-Evaluation of The acquired immunity by enzyme linked immune sorbent assay test (ELISA) for humoral immunity and for cellular immunity by Delayed type hypersensitivity test (skin test) and phagocytic index test.

4- Comparing the efficacy of these two types of vaccine candidate preparations to make a clear data about which one is batter in this mater compared with control group.