VIRULENCE GENES OF SHIGA–TOXIN PRODUCING ESCHERICHIA COLI ISOLATED FROM FOODS, ANIMALS AND HUMAN

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ANISA ABD-ALLAH AWAD SALEM

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SUERVISOR'S COMMITTEE

Prof. Dr. Abbas Amin Ahmed

Professor of Food Hygine

Fac. Vet. Med., Alexandria University

Prof. Dr. Samy Abd-El-Salam Khalil

Professor of Microbiology S.A. Khalil

Fac. Vet. Med., Alexandria University

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Prsented by
Anisa Abd-Allah Awade Salim
B.Vet.Sci. Fac0 Vet.Med.Alexandria

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Examinar Committee: **Approved**

Dr. Ahmed Mohamed Ammer
Prof of Microbiology
Fac.of vet. Med. Zagazig Univ.

Dr. Helmy Ahmed Torky
Prof. of Microbiology
Fac. of Vet. Med. Alexandria Univ.

Dr. Abbas Amin Ahmed
Prof. of Food Hygiene
Fac.of Vet.Med. Alexandria Univ.
(Supervisor)

Dr. Samy Abd El-Salam Khalel
Prof. Of Microbioligy
Fac. of Vet. Med. Alexandria Univ.
(Supervisor)

S. A. Khalil

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بسم الله الرحمن الرحيم

وما أوتيتم من العلم إلا قليلاً

صدق الله العظيم

سورة الإسراء الآية رقم ٨٥

Dedicated to

To Memory of My Father

To My mother

To My husband

To My daughter (Mariam)

To My Sisters

and

To my brothers

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ACKNOWLEDGEMENT

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INTRODUCTION

1-INTRODUCTION

Diarrhoea is a clinical entity causes serious economic losses as it may lead to high mortality, weight loss or even late growth in different animals and even in human. It is caused by a combination of many risk factors ***(Bastawerous et al., 2001, El-Ged et al., 1994).***

Diarrhoea can be attributed to infection with a single agent (in very young or stressed animals) or more commonly to multiple agents. Its severity depends partially on non-infective contributing factors and on the nature of involved organism ***(Tzipori, 1981).*** Several bacterial species may be involved in diarrhoea and losses of neonatal lambs.

The most important is being certain strains of ***Escherichia coli*** that possess virulent factors as well as and also other members of ***Enterobacteriaceae***. These pathogens are responsible for great mortality and various morbidity changes and at the same time constitute a hazard to public health ***(Orden et al., 2000).***

In Egypt, diarrhoea continues to be the first cause of mortality , which ranged between 27.4 % to 55.5 % of the total deaths in young calves ***(Ahmed, 1980).***

E. coli was the main cause of diarrhoea affecting newly born calves younger than one week ***(Jayppa et al., 1984).*** Moreover, ***E. coli*** was the most important organism incriminated as the causative agents of diarrhoea in preweaned calves ***(Asma et al., 1996).***

Higgns et al. (2005) found that diarrheogenic ***Escherichia coli***, which include the enteropathogenic ***E. coli*** and the enterohaemorrhagic ***E. coli*** are a significant cause of diarrhoea l disease among infants and children in both developing and developed areas.

There were four major categories of diarrhoeagenic ***E. coli***, namely: entertoxigenic ***E. coli*** (ETEC), enteroinvasive ***E. coli*** (EIEC), enteropathogenic ***E. coli*** (EPEC) and enterohaemorrhagic ***E. coli*** (EHEC). These categories of ***E. coli*** differ in their epidemiology and pathogenesis and their O : H serotypes ***(Levin, 1987).***

ETEC was well recognized as an important cause of neonatal diarrhoea among calves ***(Moon et al., 1978; Zeman et al., 1989 and Quinn et al., 1994).*** Also, ETEC isolated from calves was known to possess K99 (F5) adhesion antigen and to produce stable toxin (ST) but not labile toxin ***(Acres, 1985; Mainil et al., 1986; Holland, 1990 and Wary et al., 1993).***

Mellmann et al. (2005) reported that Enterohaemorrahagic ***Escherichia coli*** (EHEC) cause most cases of the haemolytic uraemic syndrome (HUS) worldwide.

Meat products may be contaminated with ***E. coli*** from food handlers, utensils, air, soil and water as well as inadequate hygienic circumstance during manufacturing, packing and marketing of these products ***(Frazier and Westhoff, 1978).***

E. coli biotype I was detected at all stages of meat handling and recognized as an indicator of direct or indirect fecal contamination of meat ***(Stiles and Lai-King, 1981).***

Many researchers concluded that EPEC isolated from different meat products was incriminated in different diarrhoea and gastrointestinal outbreaks in adult human ***(Marier et al., 1973 and Edelman and Levine, 1983).***

Shiga toxin-producing ***Escherichia coli*** (STEC), especially of serotype O157 : H7 is considered one of the most important emergent zoonotic food borne pathogens, constituting a world-wide public health problem either in the form of individual cases of infection or outbreaks ***(Leclercq et al., 2001).***

STEC infection in man has been associated with consumption of foods of animal origin especially undercooked beef or raw milk including cow's, goat's and ewe's milk ***(Little and DeLouvois , 1999; Rubin et al., 1999 and Stephen and Kuhn, 1999).***

Epidemiological investigators revealed that different species of animals may act as a reservoir of ***E. coli*** O157 : H7 ***(Nelson et al., 1998).*** ***E. coli*** O157 : H7 was a dominant VTEC serotype in many parts of the world ***(Karamali, 1989).*** In addition, ***Escherichia coli*** O157 : H7 was isolated from faeces of young cattle in dairy farms associated with two cases of haemolytic uraemic syndrome (HUS) ***(Wells et al., 1991).***

Polymerase chain reaction (PCR) technique has the advantage that it is readily applicable to large number of isolates, in contrast to classic methods such as agglutination, infant mouse, legated swine intestine and cell culture assays ***(Rodriguez, 1997).***

PCR is a valuable and sensitive method for determining the virulence factors of ***E. coli*** strains and seemed to give good results in epidemiological investigation of diarrheogenic ***E. coli*** ***(Osek et al., 1999).***

The aim of this study was:-

The objective of this study was to determine the prevalence rate and virulence character associated with ***E. coli*** isolates through:-

1-Isolation of ***E. coli*** from animals, human and foods.

2-Detection of Shiga toxin producing ***E. coli*** by PCR.

2- REVIEW OF LITERATURE

2-I- Pathogenicity of *E. coli:*

Smith and Lingood (1972) reported that enterotoxin produced by calf and lamb enterotoxigenic ***Escherichia coli*** (ETEC) strains was plasmid controlled. They also found that this plasmid was transmissible. They studied the pathogenicity of ETEC originating from calves and lambs by calf gut loop system. They found that calves and lambs were only susceptible to heat stable enterotoxins (STS) but not to heat labile enterotoxins (LTS) produced by ***E. coli*** of porcine origin. They found that enterotoxins produced by lamb enteropathogenic ***E. coli*** strains were plasmid controlled. They also found that common K antigen (K99) was controlled by transmissible plasmid and the adhesion antigen (K99) was very important in the pathogenesis of ***E. coli*** diarrhoea among lambs. ETEC isolated from diarrhoeic lambs produced only ST.

Jensen and Swift (1982) cited that colibacillosis was characterized by gastroenteritis or septicemia. The disease was caused by specific strains of ***E. coli*** and colibacillosis of lambs occurred among flock lambs where crowding and unsanitation prevailed. They reported that all breeds and sexes of lambs were susceptible to the disease but lambs of 2 – 3 days old more commonly developed the enteric form. The disease was widely spread and may develop during season, but the incidence was high among lambs born during winter and early spring. ***E. coli*** of serotype O78 : K80 was a usual cause of colibacillosis among lambs. Morbidity was high and the mortality rate varied from 15 – 75 % in affected animals.

Karamali et al. (1983) stated that EHEC is enteropathogen which is neither invasive nor enterotoxigenic . It was mentioned that EHEC O157 : H7 strain had emerged as an enteric pathogen of public health importance in Canada and the United States . Multiple reports of the infections were in the form of outbreaks of hemorrhagic colitis, haemolytic uraemic syndrome and diarrhoea in nursing Dames, day core centers and schools.

Tzipori et al. (1983) cited that rotavirus and enterotoxigenic ***E. coli*** were capable of inducing diarrhoea among lambs. They found that dual infections tended to lengthen the period of age of susceptibility and increased the severity of gastroenteritis.

Cavalieri et al. (1984) showed that the virulence of ***Escherichia coli*** was multifactorial and certain properties were associated primarily with virulent strains, one of these was the ability to produce a haemolysin. By far, most ***E. coli*** in the intestine do not have this capability, meanwhile there was a high percentage of extra intestinal isolates producing haemolysin and therefore it has been proposed to be a virulence factor.

Bohach and Snyder (1985) reported that ***E. coli*** α-haemolysin was a protein and lysis erythrocytes from several species of animals in vitro the α-haemolysin was believed to be a virulence factor.

Borczyk et al. (1987) declared that dairy cattle had been identified as a reservoir of ***E. coli*** O157 : H7. The organism was isolated on two occasions from the faeces of young animals of herds associated with cases of haemolytic uraemic syndrome in children following consumption of raw milk.

Doyle and Shoeni (1987) isolated ***E. coli*** O157 : H7 from unpasteurized milk associated with outbreaks of haemolytic colitis or haemolytic uraemic syndrome (HUS0 in humans. The milk sample was taken from the bulk tank of a farm from which ***E. coli*** O157 : H7 had been isolated from the faeces of heifers.

Niazi and Refai (1988) cited that strains of enterotoxigenic ***E. coli*** are known to produce heat labile enterotoxins (LT) that are antigenic and similar to Cholera enterotoxin

and/or heat stable enterotoxin (ST) that are of small molecular weight and are non antigenic.

Doram (1988) demonstrated the danger of food poisoning from ***E. coli*** serotype O157 : H7 due to many reports of different outbreaks. One outbreak of haemorrahagic diarrhoea was reported in Wisconsin during which 52 persons, attended abuffet, were affected. A previous outbreak of ***E. coli*** O157 : H7 poisoning was traced to under cooked meat and reports of diarrhoea were attributed to consumption of unpasteurized milk, from which ***E. coli*** O157 : H7 was isolated. Unfortunately, the serotype was also isolated from beef pork and lamb meat.

Zeman et al. (1989) cited that ETEC was one of the three principle causes of calf scour, occurring most often during the first week of life. The disease can occur in calves up to three-weeks old, but after one week, it is usually associated with other enteric infections such as rotavirus or Coronavirus. They added that ETEC was the most important pathogenic type involved in neonatal calf scours and should not be confused with other less common types of ***E. coli.*** The pathogenicity of ETEC depends on two main virulence factors, K99 (F5) and heat stable enterotoxin (STa). STa binds to receptors on villose obsorptive cells, which induces hypersecretion resulting losses of electrolytes, bicarbonate and fluid. Regarding the morbidity of ETEC infection among calves, they showed that it varies greatly from 10 – 75 % but usually near 30 % with mortality rates varying from 5 – 25 %. None ETEC intestinal pathogens have been identified as enterohaemorrhagic ***E. coli*** (EHEC) based in part on their ability to produce verotoxins.

Holland (1990) stated that bacteria can mediate diarrhoea by means of , i. invading the intestinal mucosa and eliciting an inflammatory response that mediate hypersecretion through prostaglandin and other products of inflammation, and ii. Destroying villous absorptive epithelial cells and thus causing amalabsorptive diarrhoea. He added that ETEC, EPEC and EHEC were the diarrhoeagenic types of ***E. coli*** occurring in calves and lambs , but ETEC was recognized as the important atielogical agent of diarrhoea. These organisms should not be confused with rare EPEC and EHEC that cause the less common diaerrhoeal syndrome. Also, ETEC must have specific virulence factors to induce diarrhoea among calves and lambs.

Two of the most prominent factors are (i.) expression of fimbrial antigen especially K99 that enables the bacterium to attach to intestinal cells and (ii) elaboration of STa which influences intestinal secretion of fluid through increased cyclic GMP, in addition , he stated that ETEC isolated from calves and lambs was limited to a few serotypes or serogroups especially O8, O9 ad O101.

Janke et al. (1990) described a form of ***E. coli*** infection in 60 calves from 59 farming operations. ***E. coli*** that was responsible for that infection principally colonized the colon, inducing a destructive lesion and described as attaching and effacing lesions. They observed haemorrahagic enterocolitis or blood in the faces of 46 % of calves from farms. The average age of affected calves was 11.8 days . 44 (73.3 %) of calves were infected with other enteropathogenic including cryptosporidia, rotavirus, coronavirus, bovine viral diarrhoea virus, coccidian and ETEC., and later was isolated from 6 (10 %) calves. Verocytotoxin producing ***E. coli*** was recovered from 31 out of 46 calves, 17 of them produced high toxin titer (320 to 1640) and the most predominant serotype was O111 : NM – as it accounted for 53 % of the isolates. Meanwhile, O157 : NM was not identified. They added that infection with attaching and effacing ***E. coli*** should be considered in young calves with haemorrhagic colitis or when blood is observed in the faeces.

Montenegro et al. (1990) reported that Verotoxin-producing ***E. coli*** (VTEC), was the causative agent of haemorrhagic colitis, haemorrhagic uraemic syndrome and thrombocytopenic purpura in human.

Booth and Rowe (1993) stated that ***E. coli*** O157 : H7 infections in human may be acquired via contact with infected animals, infected humans and consumption of contaminated food or water. They also reported a case of laboratory acquired infection in a technican.

Duhamel et al. (1992) described the incidence of naturally occurring enteric colibacillosis produced by enterohaemorrhagic Shiga-like toxin I producing ***E. coli*** (EHEC) caused severe fatal diarrhoea in a 2 months old goat.

Quinn et al. (1994) cited that ***E. coli*** , Salmonella Sp., Clostridium perfringenes and rotavirus were implicated as casuses of diarrhoea among lambs. Colibacillosis was most commonly seen in neonatal lambs in crowding lambs sheds and characterized by acute diarrhoea , septicemia and sudden death may occur.

Wray et al. (1993) mentioned that the main clinical signs associated with ***E. coli*** infection in calves were mainly limited to intestine causing enteric colibacillosis or neonatal diarrhoea or may be manifested as septicaemia (Colisepticemia or systemic colibacillosis) or toxaemia (Colibacillary toxemia). The diarrhoea developed within the first few days of birth as profuse watery diarrhoea , white or yellowish and rancid, the rancid faecal material accumulated in the hind limb of animal, acidosis, dehydration and death occurred within few days and in all mild cases, the animal recovered spontaneously.

Radostits et al. (1994) mentioned that enteric colibacillosis occurs in colostrums deprived animals and is caused by colonization and proliferation of enteropathogenic ***E. coli*** which produces enterotoxin and causes varying degrees of diarrhoea , acidosis and dehydration. While single infection occurs commonly , multiple infections with enteropathogenic ***E. coli*** and viruses and other agents are more common. They added that the major virulence factors attributing to ETEC in calves are the K99 aheshion antigen and the heat stable enterotoxin (ST). Some diarrhoeic calves may be infected with VTEC. Also, attaching and effacing ***E. coli*** of serotype O111, O5 and O26 were found with increased frequency. These strains produce verotoxin and a characteristic attaching and effacing lesions. Diarrhoea and dysentery may be due to haemorrhagic colitis in calves of 2 – 5 weeks old.

Litalien et al. (1999) cited that the exact mechanism for the syndrome (HUS), however, remains speculative. Endothelial injury was considered the primary pathogenic event in diarrhoea associated US. Moreover, an acute inflammatory response in STEC infection including leukocytes and inflammatory mediators was also suggested to play an important role in the pathogenesis of STEC induced HUS by enhancing the effect of Shiga toxins produced by the organisms.

Chapman (2000) reported that cattle faeces are an important source of ***E. coli*** O157 : H7 in the environment and contact with contaminated faeces a risk to human.

Jansson et al. (2001) reported that in 1997, a Swedish dairy farm was implicated in human case of verotoxigenic ***E. coli*** (VTEC) infection. They found ***E. coli*** O157 : H7 in faecal sample from the human cases and in faecal samples from calves kept on pastures and calves kept in doors during the summar.

Karpman et al. (2001) mentioned that Shiga-toxin producing ***E. coli*** is associated with wide spectrum of clinical manifestations including non-specific diarrhoea , haemorrhagic colitis and life threating haemolytic uraemic syndrome (HUS) which leads to acute renal failure in children and thrombocytopenic purpura. All of which are related to

adherence of the pathogen to intestinal tract lining followed by production of one or more verotoxins which are implicated in vascular endothelial damage observed in haemorrahgic colitis and HUS patients. Shigatoxins STX have been specifically implicated as a causal factor for HUS because cases with HUS were found to be associated with STX-producing strains, the toxin has been identified in the kidney of patients with the syndrome; and the toxin was found to be cytotoxic for renal endothelial and epithelial cells.

Stephan et al. (2004) cited that enteropathgenic ***Escherichia coli*** (EEC) mainly caused infantile diarrhoea , represented one of at least six different categories of diarrhoea genic ***E. coli*** with corresponding distinct pathogenic schems.

The mechanism of EPEC pathogenesis is based on the ability to introduce the attaching and effacing (A/E) lesions and intimate adherence of bacteria to the intestinal epithelium,. Cattle seemed to be a reservoir of O157 : H45 EPEC strains which are described in association with human diseases. Therefore, these strains appear to play a role as foodborn pathogens and has to be considered and evaluated in view of food safety aspects.

Karmali (2004) mentioned that ***E. coli*** O157 : H7 is the prototypic enterohaemorrhagic ***E. coli*** (EHEC) which produces potent cytotoxins known as Shiga toxins (SLT) or Shiga toxins (STX). Shiga toxins are translocated from the bowel to the circulatory system and transported by leucocytes to capillary endothelial cells in renal glomeruli and other organs.

Brooks et al. (2005) cited that Shiga-toxin producing ***Escherichia coli*** (STEC) O157 : H7 is a well recognized cause of bloody diarrhoea and haemolytic uraemic syndrome (US), non O157 : STEC infections were most frequent during the summer and among young persons (median age, 12 years, interquartile range, 3 – 37 years). STX (2) was strongly associated with an increased risk of HUS, and eae was strongly associated with an increased risk of bloody diarrhoea . Non O157 SEC caused severe illness that is comparable to the illness caused by STEC O157. Strains that produced Shiga toxin2 are much likely to cause HUS than are those that produce Shiga toxin I aone.

2-2.Incidence of Escherichia coli:-

2-2.I-Incidence of *Escherichia coli* in animals:-

Khera and Dhanda (1963) found that out of 67 calves dead with pure systemic infections, 35 were ***E. coli*** infections.

Mayer et al. (1964) reported an annual losses of 10 % of the calves due to ***E. coli*** infections.

Yalcin et al. (1969) isolated 1190 bacterial strains from calve that died from diarrhoea and septicaemia, 58.5% of these strains were pure culture of E. coli, 8.7 % were colostridia and 57.5 % were mixed cultures of the two organisms.

Amrousi et al. (1972) isolated ***E. coli*** in an incidence of 30% from calves of 4 and 30 days old suffering from diarrhoea .

Glantz, et al. (1972) isolated a particular strain of ***E. coli*** from 11 diarrhoeic calves of 5 – 10 days old.

Singh and Singh (1972) ***isolated E. coli*** in an incidence of 18.3% from calves showing severe gastroenteritis.

Alikaer and Zeroza (1973) isolated 44 strains of ***E. coli*** which belonged serologically to seven groups from diarrhoeic dead calves.

Oxender et al. (1973) revealed that calf mortality due to ***E. coli*** was about 17.7% between birth and 60 days of age. Deaths between 6 months up to 9 months reached 8.5%. Mortality ranged from 16.1% for herds less than 60 cows to 34.9% for herds of more than 200 cows.

Prohoszka (1973) studied factors predisposing newborn calves to mass outbreaks of ***E. coli*** enteritis. He found that entry of ***E. coli*** into the general circulation and intestinal organs occurred through intestinal epithelium.

Speicher and Hepp (1973) showed that the annual calf mortality due to ***E. coli*** averaged 13.5%. Winter and Summer death losses were 17.1% and 10.3% respectively. Mortality increased as herd size increased from 9.7% for herds of less than 25 cows to 16.6% for herds of more than 85 cows.

Boyd et al. (1974) survied the incidence of neonatal diarrhoea in calves during four consecutive Winters due to ***E. coli***. The mortality from neonatal diarrhoea due to ***E. coli*** was 12.0%.

Damodaran and Sundararaj (1974) examined bacteriologically 2835 calf deaths with intestinal disorders. ***E. coli*** caused 29.9% of such deathes. ***E. coli*** was seen in the intestinal contents of 45.2% of dead calves.

Ranatunga (1974) recorded that 850 (15.6%) of calves died before 6 months of age was due to ***E. coli*** infection. The overall postnatal mortality with Colibacillosis was higher in males (17.21%) than in females (15.21%). ***E. coli*** mortality was 4.5% up to one month, 3.5% between 1 and 2 months, 2.9% between 2 and 3 months, 1.4% between 4 and 5 months and 1.8% between 5 and 6 months of age.

Fwasher and Martinez (1975) noticed that from 11 out of 26 calves studied, diarrhoeic ***E. coli*** was the predominant organism isolated from faecal swabs.

Fwasher and Martinez (1975) added that ***E. coli*** was isolated from 5 dieing diarrhoeic calves and 3 surviving diarrhoeic calves.

Farid et al. (1976) isolated ***E. coli*** serogroups O117, O115, O35, O137, O101, O9, O8, O15, O26, O119, O86, O111, O126 and O125from buffalo and Friesian calf faecal samples from farms with problems of enteritis in Egypt.

Sivaswamy and Gyles (1976) examined 300 strains of ***E. coli*** to determine the prevalence of enterotoxigenicity among ***E. coli*** isolated from faces of scouring and normal calves. They found that 24.5 % of ***E. coli*** isolated from scouring calves were enterotoxigenic and only 1 % of those isolated from normal calves were enterotoxigenic. ***E. coli*** of bovine origin produced an enterotoxin that resembles the ST rather than LT form of enterotoxin produced typical pig ETEC strain.

Al-Khayyat et al. (1977) reported that out of two hindered newlyborn calves, one hundred were suffered from diarrhoea. Bacteriological examination revealed that ***E. coli*** was isolated with incidence of 68 %.

Isaacson et al. (1978) isolated ***E. coli*** strains at the rate of 53 % from from 1 – 17 days old calves with diarrhoea , as well as 15 % from calves without diarrhoea .

Farid et al. (1979) isolated 276 ***E. coli*** strains from 316 fecal samples collected from newly born-apparently healthy buffalo-calves at the rate of 87.3 %.

Svastova (1980) isolated 2112 ***E. coli*** strains from 1182 calves suffering from enteritis and found that 569 strain were classified into 11 antigenic groups (O15, O139, O141, O8, O197, O101, O2, O147, O78 and O115).

Talavera coronel (1981) recovered 33 strains of ***E. coli*** belonging to O-groups 45, , 18, 3, 21 and 149.

Valente et al. (1982) found that out of 137 ***E. coli*** isolates recovered from calves with diarrhoea 28 (20 %) were O78, 21 (15 %) were O15, 21 (15 %) were O8 and 20 (14.5 %) were O101.

Tripathi and Soni (1983) isolated 33 strains of ***E. coli*** from 50 diarrhoeic newlyborn calves. They belong to groups O11, O15, O55, O40, O21 and O26.

Jayappa et al. (1984) revealed that ***E. coli*** was the main cause of diarrhoea affecting newly born calves younger than one week.

Hall et al. (1985) recovered ***E. coli*** from cases of dystentry syndromes which occurred among calves of 1 to 12 days old.

Krogh et al. (1985) Stated that there were mixed infection occur associated with calves diarrhoea when 191 cases were examined. They found that enterotoxigenic strain of ***E. coli*** (ETEC) was isolated from 4 cases and septicemic ***E. coli*** from 2 cases.

Adetosoye (1986) recovered 17 strains of ***E. coli*** from diarrhoeic calves belonging to O-groups 26, 15 and 17.

El Gannam and Sidorv (1986) reported that among 44 ***E. coli*** strains isolated from calves, 34 (77.3 %) were haemolytic.

Zajicek et al. (1986) recorded Simultaneous isolation of ***E. coli*** and Cryptosporidum in 11.5% of calves with catarrhal haemorrhagic enteritis aged 2 – 30 days suffering from digestive disturbances.

Reynolds et al. (1986) examined fecal samples from diarrhoeic calves. They found that enteropathogenic ***E. coli*** detected in 9 of 310 calves (3 %).

Walter et al. (1986) isolated enteropathogenic ***E. coli*** at a percentage of 41 % from randomaly selected Holstein dairy farm in South Western Ontaro.

Morris et al. (1987) mentioned that ***E. coli*** could be isolated from calves at a percentage of 12.1 % . They reported hat 20 calves of 46 diarrhoeic calves suffered from mixed infection with ***E. coli*** and ***Salmonella***.

Kovaler et al. (1987) found that eneropathogenic strains of ***E. coli*** isolated from diarrhoeic calves were belonged to serovars O119, O26 ad O127.

Oliveir et al. (1989) collected fecal samples from 1720 healthy and diarrhoeic buffalo calves up to 4 months old. They found that ***E. coli*** was isolated from 95.4 %

Bulte et al. (1990) were able to identify 80 verotoxin producing ***E. coli*** strains out of 2100 tested strains using a specific gene proble. The tested ***E. coli*** (2100) strains were isolated from faeces of cattle and pigs. ***E. coli*** O157 : H7 strain was predominant among the verotoxic strains in addition to the serotype O22 : H8, O82 : H8 and other strains belonging to the O. groups 39, 91, 113 and 126.

Dorn and Angrick (1991) investigated ***E. coli*** O157 : H7 strains from different sources using different verotoxin DNA specific probes. The obtained results indicated that genetic variations are existing among different strains of ***E. coli*** O157 : H7 and there was no similarity between strains of the same sources. However, some strains of bovine origin hybridized in a manner similar to strains of human origin.

Uysal et al. (1992) studied 74 isolates of ***E. coli*** from cases of calf diarrhoea . They found that the commenest somatic serotypes were O141 (14 %) , O114 (11 %), O101 (9.5 %) and O73 (9.5 %).

Wolk et al. (1992) found that ***E. coli*** strains isolated from 60 sick calves with diarrhoea were belonged to 0 serogroups 101, 9, 8 , 20 and 56.

Viring et al. (1993) stated that ***E. coli*** could be isolated from diarrhoeic fecal samples of calves at a percentage of 11.5 %.

Manaa et al. (1993) carried out an investigation on 40 diarrhoeic and 20 clinically healthy calves. They isolated ***E. coli*** with a percentage of 80 %.

Mahmoud (1993) isolated enteropathogenic ***E. coli*** EPEC from 25 % of diarrhoeic calves.

Caprioli et al. (1993) examined faecal samples from 153 veal calves and 18 dairy cows in north Italy for the prevalence of verotoxin producing E. coli. They found that 8 isolates out of 71 (4.6 %) from healthy cows and calves were positive for VTEC and 5 (7.7 %) isolates from diarrhoeic calves were positive for VT. All the isolates from diarrhoeic calves produced VT and all of these isolates were not belonging to serotype O157 : H7.

Radostitis et al. (1994) stated that ETEC was isolated from the faeces of approximately 35 % of the diarrhoeic lambs. They cited that the most prevalent causes of diarrhoea among newborn lambs and goat kids were ETEC, clostridium perfringenes types B and D and salmonella species.

Chapman et al. (1994) isolated 84 (8.2 %) strains of ***E. coli*** O157 out of 1024 rectal swabs taken from dairy cattle over a 4 month period.

Garber et al. (1995) reported that ***E. coli*** O157 : H7 was isolated from 31 out of 965 dairy calves in 64 farms. Sample prevalence of ***E. coli*** O157 : H7 serotype was 1.4 % in calves less than 8 week-old and 4.8 % in calves of the age 8 weeks or older. Calves were three times more likely to shed ***E. coli*** O157 : H7 after than before weaning.

Mona (1995) isolated ***E. coli*** with 33.33% from 105 fecal samples collected from diarrhoeic buffalo-calves. The most common serotypes were O55 : B5 (24 %) O26 : B6 (18 %), O86 : B7 (12 %), O119 : B 14 (10 %) , O111 : B4 (8 %), O 125 : B 15 (6 %), O128 : B12 (6 %) and O127 : B8 (4 %) in addition to 3 (6 %) untypable strains of E. coli.

Shoning and Sagartz (1995) found that enteritis was responsible for mortality of 5.11% of 8 – 28 days old lamb. ***E. coli*** was the only aetiological agent found in lambs of this age that died from enteric disease.

Steiner et al. (1997) carried-out bacteriological examination on fecal and blood samples collected from diarrhoeic and died calves. They isolated ***E. coli*** at a percentage of 42 % from fecal samples.

De La Funte et al. (1998) examined 218 fecal samples collected from diarroeic calves calves at age of 1 – 30 days old. They could isolate ***E. coli*** at a percentage of 11.9 %

Perez et al. (1998) carried out a study on 194 faecal samples from calves with clinical signs of diarrhoea and 186 samples from calves without diarrhoea for the presence of bacteria. ***E. coli*** were detected in 94% of all samples.

Garcia et al. (2000) found that the percentage of enteropathogenic bacteria was 16.7% for ***E. coli.***

Samer (2001) revealed that only 4.91 % (9/140) of ***E. coli*** isolates from diarrhoeic animals were α-haemolysin producing ***E. coli.***

Haggag and Khaliel (2002) examined bacteriologically 104 fecal samples from diarrhoeic calves. They isolated ***E. coli*** at a percentage of 82 %.

Entsar (2004) cited that ***E. coli*** was the most predominant bacterial pathogen associated with calf diarrhoea . ***E. coli*** could be isolated from faecal samples of examined calves at a percentage of 81.25 %. The isolation rates of ***E. coli*** from fecal samples of examined calves in relation to age were 86.25 % (1 – 30 days old), 72.5 % (31 – 60 days old) and 80 % (61 – 90 days old).

Gaber (2004) found that 10.2 % (5 / 49) of ***E. coli*** isolates from calves were α-haemolytic.

2.2.2.Incidence of *E. coli* in foods:

Mello and Amaral (1940) examined 30 samples of market cheese made from raw milk. 75.56 % of isolated microorganisms were identified as Escherichia coli.

Manzello and Esterez (1965) could isolate ***E. coli*** (O111 : B4) from 17 out of 250 samples of raw milk.

Hall et al. (1967) could isolate E. coli, Klebsiella aerogenes and intermediate types from examined samples of cheese.

Moursy and Nasr (1964) isolated ***E. coli*** by 95 % from cheese.

Maida et al. (1970) cited that incidence of ***E. coli*** isolation constituted 11.2 % from raw milk.

Singh et al. (1970) reported that incidence of enteropathogenic ***E. coli*** was 19 % in raw milk, and 16 % in market milk samples collected from a local market.

June et al. (1953) mentioned that certain serotypes of ***Escherichia coli*** may give rise to outbreaks of meat borne gastroenteritis for some consumers.

Floyd and Blakmore (1954) isolated human enteric pathogen enteropathogenic ***Escherichia coli*** (EPEC) from 7 (2.8 %) out of the 250 examined meat samples which were collected from Cairo shops.

Freeman (1960) reported that ***Escherichia coli*** and Coliforms are indicators for the sanitary quality of foods. As the presence of large numbers of these organisms indicates sanitary neglected measures during preparation of meat leading to spoilage loss of quality or danger to health.

Thomson et al. (1961) cited that certain ***Escherichia coli*** are incriminated in cases of food poisoning and gasterointestinal disorders among consumers, as well as in severe diarrhoea .

Roushdy (1971) found that the incidence of ***Escherichia coli*** was 24 % from examined minced meat samples.

Duitschaever et al. (1973) detected ***Escherichia coli*** in 39.8 % of exmined samples of various types of raw refrigerated ground beef meat.

Miltere et al. (1973) isolated ***E. coli*** was by incidence rate of 25 % from milk.

Bogdanowicz and NockIewwicz (1973) examined bacteriologically 240 samples of raw milk taken during one year from collection tank and found that the percentage of ***E. coli*** 98.34 %. Pathogenic types O111 : B4 and O26 : B6 could be identified.

Deucn et al. (1974) isolated 18 ***E. coli*** strains from raw milk samples, 7 out of which could be serologically typed s O9 : K9.

Nenkov (1974) could isolated enteropathogenic ***E. coli*** belonging to 10 serotypes) from 4 out 123 samples of milk examined. He isolated Enteropathogenic ***E. coli*** from 2 samples out of 18 samples of fresh cheese.

Psassis et al. (1974) found that during the examination of 251 food samples including 99 cheese samples, 157 were found to be contaminated with E. coli.

Singh and Ranganathan (1974) cited that the predominant serotypes of ***E. coli*** isolated from milk were O55 : B5 , O126 : B 16, O26 : B6 and O127 : B8. They reported that 2 out of 15 of examined cheese samples were contaminated with ***E. coli.***

Fantasia et al. (1975) reported that about 10 % of cheese associated with diarrhoeal disease contained enteropthogenic strains of ***E. coli.***

Moustafa et al. (1975) examined bacteriologically 64 milk samples, and found that ***E. coli*** could be isolated from 21 % of examined samples.

El-Bassiony (1977) examined 100 smples of fresh kariesh cheese. He isolated ***E. coli*** by incidence rate of 67 %.

Foster et al. (1977) examined 150 samples of ground beef. The most common isolated micro-organisms were ***Escherichia coli***, Klebsiella species and enterobacter species.

Singh and Ranganathan (1977) found that 30 out of 129 samples of raw milk containing 10 enteropathogenic ***Escherichia coli.***

Adel (1978) isolated ***E. coli*** by incidence rate of 36 % from raw milk.

Moustafa (1978) isolated ***E. coli*** from milk by incidence rate of 70 %, 70 % and 73.33% respectively from street vendors, dairy shops and dairy farm.

Moustafa (1978) examined bacteriologically milk samples collected from dairy farms in Assuit province, ***E. coli*** existed in 73.33 % of examined samples.

Saudi (1978) isolated ***E. coli*** by incidence rate of 36 % from raw milk.

Frazier (1979) cited that ***E. coli*** may also be the causal organisms in appendicular abcess, peritonitis and cholecystitis.

Mohamed (1979) isolated ***E. coli*** by incidence rate of 34.27 % from cheese.

Shelaih (1979) isolated 34.92 % ***E. coli*** from kariesh cheese samples.

Pandey and Mandel (1980) mentioned that ***E. coli*** was found in 10 % out of 40 samples of raw milk.

Ahmed (1980) examined Kariesh cheese for the presence of ***E. coli*** and Enteropathogenic ***E. coli*** (EEC). He isolated 48 strains of ***E. coli*** from examined cheese samples, out of these strains 26 were found to be EEC.

Ergullu (1982) reported that ***E. coli*** was found in 10.8 % of examined milk samples.

Cook et al. (1983) isolated ***E. coli*** from 35 cheese samples.

Hassan (1983) isolated ***E. coli*** by incidence rate of 82.9 % from raw milk and 81 % from cheese.

Nagah (1984) isolated ***E. coli*** by incidence rate of 24 % from raw milk and 22 % from kariesh cheese.

Youssef et al. (1984) isolated members of enteric pathogen from raw minced meat samples collected from Assuit city markets. They isolated Enteropathogenic ***E. coli*** (EPEC) by incidence of 10 %.

National Academy of Sciences (NAS) (1985) stated that improper cooking and handling of raw meats in homes and food services establishment is one of the main reasons for foodborne illness. Moreover, poor personnal hygiene, inadequate sanitation and crowded living conditions are important factors in the spread of foodborne disease.

Darwish et al. (1986) reported that the incidence of ***Escherichia coli*** in examined samples of minced meat was 4 (20 %).

Emam (1986) isolated ***E. coli*** from milk by incidence rate of 70 % (milk from market), 66.6 % (milk from dairy farm) and 75 % from kariesh cheese.

Mousa and Yassien (1987) examined 150 samples from 30 carcass slaughtered in abattoir. The highest isolates were proteus species (41.7 %) followed by ***Escherichia coli*** (23 %).

Ahmed et al. (1988a) isolated ***E. coli*** from 80 % of examined kariesh cheese.

Ahmed et al. (1988b) examined 100 samples of Egyptian soft cheese for enteropathogenic ***E. coli*** (EEC). ***E. coli*** existed in 84 % of kariesh cheese samples.

El-Mosslami et al. (1988) evaluated 100 beef samples collected from both markets and butcher's shops. They found that incidence of ***E. coli*** was 28.9% and 41.8% respectively.

Niazi and Refai (1988) isolated ***Escherichia coli*** strains from 50 raw minced meat samples collected from different markets and shops in Giza and Cairo cities. ***Escherichia coli*** isolates were detected in 32 sdamples and identified as ***Escherichia coli*** biovr I.

Iman (1989) examined 25 samples from minced meat. He found that the incidence of ***Escherichia coli*** was 84 % but the incidence of enteropathogenic ***Escherichia coli*** was 12 %.

Amal (1990) isolated ***E. coli*** from kariesh cheese with incidence rate of 42.5 % (17 out of 40) and EEC with incidence rate of 94.1 % (16 out of 17).

Eman (1990) examined 25 samples from fresh minced meat. He found that incidence of ***E. coli*** was 48 %. The isolated enteropathogenic ***E. coli*** serovar from minced meat were O124 : K72 (2 strains), O44 : K74 (25 strain) and O127 : K63 (one strain).

Faisal (1993) isolated ***Escherichia coli*** from 50 raw meat samples with incidence rate 32 %. He cited that the incidence of Enteropathogenic ***Escherichia coli*** strains was 50 % of the isolated ***E. coli*** strains.

Zhao et al. (2001) isolated ***E. coli*** by incidence rate of 19 % from raw meat.

Gonzalez Garcia (2002) cited that EHEC was responsible for many outbreaks of bloody diarrhoea caused by contaminated foods. The most important serogroups among EHEC are O26, O111 and O157, being O157 : H7 the most relevant serotype in foodborne outbreaks. The normal intestinal microflora of cattle was found to be the most relevant reservoir of EHEC strains.

Gillespie et al. (2003) cited that 52 % of milk samples was the most commonly reported vehicle of infection in milkborne outbreaks.

Mckee et al. (2003) cited that the incidence of VTEC was low in the dairy milk and meat samples tested, and this finding may help to explain the low incidence of VTEC reported.

O' Ferrall-Berndt (2003) cited that from 135 milk samples purchased from milk-shops, 87 % were not fit for human consumption on the basis of the minimum standard prescribed in the food stuffs, cosmetics and disinfectants. ***Escherichia coli*** was detected in 17% of milk-shop milk, 95 % of which originated from milk which was alkaline phosphate positive.

Adwan and Adwan (2004) cited that the majority of STEC (Shiga toxigenic ***E. coli***) isolated from meat samples (70.5 %) harbored both Stx1 and Stx2 genes while the others harbored either Stx1 or Stx2.

Hussein and Bollinger (2005) cited that large number of Shiga toxin-producing ***Escherichia coli*** (STEC) strains caused major outbreaks and sporadic cases of human illnesses, incluing mild diarrhoea , bloody diarrhoea , haemorrhagic colitis and the life-threating hemolytic uraemic syndrome. These illness have been traced to both O157 and

non O157 STEC. In a large number of STEC-associated outbreaks, the infections were attributed to consumption of ground beef or other beef products contamined with cattle faeces. Thus, beef cattle are considered as reservoir of STEC and can pose significant health risks to humans.

Hussein and Sakuma (2005) assessed the role of dairy cattle and their products in human infections with shigatoxin-producing ***Escherichia coli*** (STEC). A large number of STEC strains (e.g., members of the serogroups O26, O91, O103, O111, O118, O145 and O166) caused major outbreaks and sporadic cases of human illnesses. These illnesses ranged from mild diarrhoea to the life-threating hemolytic uremic syndrome.

Hussein and Sakuma (2005) cited that STEC infection was attributed to consumption of dairy products that were contaminated with animal faeces. Thus, dairy cattle are considered reservoirs of STEC and can impose a significant health risk to humans.

Caro et al. (2006) demonstrated that raw milk used in cheese making may be sporadically contaminated with ***E. coli*** strains that are potentially pathogenic for humans.

2-2-3-Incidence of *E. coli* in human:-

Pain et al. (1988) isolated STEC from children with diarrhea with rate of 3.2 %.

Griffen and Tauxe (1991) cited that the attack rate of STEC infection among children ranged from 0.1 % to 71 %.

Ramoter et al. (1995) isolated STEC from children with diarrhea with a rate of 0.6 %

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Buteau et al. (2000) isolated STEC from children with diarrhea with rate of 12 %.

Loirat (2001) isolated STEC from children with diarrhea with rate of 85 %.

Asinobi et al. (2003) cited that the prevalence of bacteriuria in children with SCA was 21.6 % compared with 15.8 % in the controls. ***Escherichia coli*** and Klebsiella species were the predominant isolates from the urine, accounting for 64.9 % and 18.9 %, respectively, of the isolates from the SCA group and 63 % and 22.2 %, respectively, in the controls.

Aslan and Bouzari (2003) found that 0.7 % of the population were infected with VTEC, however non of the isolates belonged to O157 : H7 serotype. They also found that children less than 6 years of age were at highest risk of infection with VTEC.

Savarino et al. (2003) cited that the incidence of enteropathogenic ***Escherichia coli*** diarrhoea among Egyptian children was 1.5 episodes per child per year and accounted for 66 % of all first episodes of diarrhoea after birth. The incidence increased from 1.7 episodes per child per year in the first 6 months of life to 2.3 in the second 6 months and declined thereafter.

Addy et al. (2004) cited that EPEC and cryptosporidium may be considered as important diarrhoeagenic pathogens and it is hoped that provision of palatable water and good sanitation may decrease diarrhoea s in infants caused by these agents. They cited that Enteropathogenic ***E. coli*** was the most frequently detected pathogen by incidence rate of 14.8 % of the findings in the diarrhoea l group.

Blanco et al. (2004) detected STEC strains in 126 (2.5 %) of 5054 cases of children with diarrhea, or other gastro-intestinal alterations.

Mercado et al. (2004) cited that highly virulent STEC strains are being shed by diarrhoeic calves and from farms located in a high incidence area of human STEC infection.

Wenneras and Erling (2004) assessed the importance of enterotoxigenic ***Escherichia coli*** (ETEC) as a diarrhoeal agent in developing countries. Two hundred and eighty millions episodes of diarrhoea due to ETEC were seen yearly among those aged less than five years and close to 50 million children of this age group were symptomatic carriers of ETEC. ETEC remains an important diarrhoeal pathogen among children in the developing world.

Berkely et al. (2005) isolated ***E. coli*** by incidence of 10 % among children with 60 or more days of age.

Franzolin et al. (2005) reported the frequency of different diarrheogenic ***Escherichi coli*** (DEC) categories isolated from children with acute endemic diarrhoea . ***E. coli*** isolates were investigated by colony blot hybridization with genes porobes : eae, EAF, bFpA, Stx1, Stx2, St-Ih, St-IP, LT-I. LT-II, INV, and EAEC, as virulence markers to distinguish typical and a typical EPEC, EHEC/STEC, ETEC, ETEC, and EAEC. Seven of the eight categories of DEC were detected. The most frequently isolated was a typical EPEC (10.1 %) followed by ETEC (7.5 %) and EAEC (4.2 %).

EHEC, STEC, EIEC and typical EPEC were each detected once. The strains of ETEC, EAEC and a typical EPEC belonged to a wide variety of serotypes. The serotypes of the other categories were O26 : H11 (EHEC), O21 : (STEC), O142 : H34 (typical EPEC), and O : H55 (EIEC).

Potgieter et al. (2005) cited that the frequency of isolation of ***E. coli*** was 70 %. The frequent contamination of water and food samples has important implications for the health of children from impove rished communities.

Qadri et al. (2005) cited that Enteropathogenic ***Escherichia coli*** (ETEC) that produced heat-stable enterotoxin alone was most prevalent, and 78 % of strains had colonization factors. ETEC cause epidemic diarrhoea .

Koehler et al. (2006) cited that previous studies of bacterial enteric infections suggested a disproportionate disease burden for children younger than 5 years off age. The incidence of ***Escherichia coli*** was 32.7 %. They cited that investigation of risk factors specific to this age group, review , enhancement of current prevention and control strategies for children younger than 5 years of age may reduce illness.

Sakuma et al. (2006) cited that the annual incidence of vetrotoxin-producing ***Escherichia coli*** was 2.74 / 100.000 population ; its fluctuation over time and space was associated with climate, socioeconomic, and population factors.

Vernacchio et al. (2006) cited that the most common micro-organisms identified in healthy baseline stools were a typical enteropathogenic ***Escherichia coli*** (12.2 %0) and enteroaggregative ***Escherichia coli*** (3.7 %). Each of these was no more common in diarrhoea stools.

Wierzba et al. (2006) cited that ETEC-associated diarrhoea in children was 14 % from patients with diarrhoea . ETEC did not have any clinically distinct characteristics.

2-3-Polymerase chain reaction (PCR):

Saiki et al. (1985) described the first practical application of in vitro DNA amplification, which become known as PCR. They used this technique for diagnosis of Sickle – cell anemia by amplification of ß-globin gene.

Kwok et al. (1987) applied PCR technique for identification of microorganisms for the first time. They used this technique for identification of human immunodeficiency virus (H1V).

Sambrook et al. (1989) said that, in the PCR, two oligonucleotides are used as primers for a series synthetic reactions that are catalyzed by a DNA polymerase. These primers typically have different sequences and are complomentary two apposite strands of the DNA sequence to be amplified. After heat mediated denaturation of the template DNA, the primer binds to their respective sequence (annealing) on the template DNA and a DNA polymerase synthesizes a complomentary strand in the 5' to 3' (extension). Each round of denaturation, annealing and extension is known as a cycle. Because the products of one round of amplification serve as templates for the next, each successive cycle essentially doubles the amount of the desired DNA products exponentially.

Erlich et al. (1991) described PCR as an in-vitro amplification technique for enzymatic synthesis of specific DNA sequences using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. A repetitive series of cycles involves: Template denaturation, primer annealing, and extension of the annealed primers by thermostable DNA polymerase.

Lantz et al. (1994) showed the usefulness of the PCR for detection of micro-organisms in food and other complex samples, however, was limited by the presence of substances that inhibit DNA polymerase, bind magnesium, and / or denature DNA. Sensitivity was decreased dramatically of the sample contains inhibitors; therefore, extensive sample preparation and DNA extraction steps were usually required prior to the PCR.

Olsvik et al. (1994) cited that the ability of PCR to amplify specific DNA reduces the need for the large quantities of test DNA required for the hybridization assays. In theory one copy of the target gene is sufficient for successful amplification.

Stone et al. (1994) developed a procedure to overcome the problems associated with application of PCR tp clinical samples. The procedure included cultivation of faecal samples seeded with known numbers of ***Salmonella*** organisms in pure culture and as little as 300 Fg of purified chromosomal DNA. They also, found that Rappaport vassilidis and tetrathionate broth were inhibitory to PCR, were is brain heart infusion and selenite-Cystine broth were not. PCR was able to as few a 80 CFU of ***Salmonella*** organisms in enriched culture of seeded faeces incubated for 2 hours. The sensitivity and specificity of this assay were both 100 % compared with culture results. They added that the sensitivity of detection of organisms in prepared faeces was approximately 10 folds lower than that in pure culture.

Ibrahim (1995) examined 141 ***E. coli*** isolates from diarrhoeic and healthy calves and their dams for virulence factors. By examining the isolates for SLT using Vero cell assay and PCR technique . He found that 5 (2.5 %) of the isolates were SLT producing ***E. coli***, 6 (4.25 %) of isolates were E. Hly producing ***E. coli*** and 4 (2.8%) showed α-Hly and no isolates produce CNF. The 5 SLT producing ***E. coli*** tested by Vero cells were also positive by PCR for SLT genes and non of them had LT1 or STa genes.

Schmidt et al. (1995) stated that PCR assay is more rapid, simple and highly sensitive than cell cultures assay and colony hybridization for detection of

enteroaggregative ***E. coli***. They also added that this assay could be recommended as a screening method in a clinical laboratory.

Rodriguez (1997) cited that PCR technique is widely used in veterinary research and this technique is likely to have a strong impact in the epidemiology, treatment and prevention of animal infectious diseases. The emergence of PCR, however, offers the potential to improve the laboratory-based diagnosis of pathogens.

Franckel et al. (1998) cited that a multiplex PCR was developed to identify enterotoxigenic attaching and effacing, and Shiga toxin-producing ***E. coli*** strains by amplifying genes encoding K99 and full fimtiae, heat-stable enterotoxin a, intimin, and Shiga toxins 1 and 2. This multiplex PCR was specific and sensitive. It will be useful for identification of ***E. coli*** strains which cause diarrhoea in calves.

Fratamico and Strobaugh (1998) concluded that the the multiplex PCR was a potentially powerful technique for rapid and sensitive co-detection of both pathogens in foods and other types of samples.

Osek et al. (1999) reported that polymerase chain reaction (PCR) was a valuable and sensitive method for determining the virulence factors of ***E. coli*** strains. PCR seemed to give good results in epidemiological investigation of diarrheogenic ***E. coli*** especially fimbrial and toxin (LT1, LT2, STa, STb, SLT1 and SLT2) genes.

Feng and Monday (2000) cited that PCR used now in large scale as a recent techniques for detection of virulence factors in enterotoxigenic ***Escherichia coli*** serotypes in meat and meat by products.

McKee et al. (2003) cited that amultiplex polymerase chain reaction assay was also used to detect the presence of VT1, VT2 and eae genes.

Muehlher et al. (2003) cited that 16.3 % of milk samples were PCR positive for Shiga-toxin producing ***E. coli***, 23.8 % positive for STEC isolated from tank milk.

Gaber (2004) cited that the PCR indicated SLT-II producing ***E. coli*** was 21.4 % (3/14).

Glennan et al. (2004) cited that Real-time PCR assays, based on hybridization probes and light cycler technology, were developed for VIT and VT2 genes.

Khan et al. (2005) cited that primary screening by PCR detected Stx1 and Stx2, the common virulence genes of STEC, in 18.9 % of cow faeces, 32.4 % of calf stool samples, 21.6 % of farm floor swabs and 4.5 % raw milk samples.

Rey et al. (2005) determined the prevalence, serotypes and virulence genes of Shiga-toxin producing ***E. coli*** (STEC) strains isolated from different dairy products with the purpose of determining wether dairy products represent a potential source of STEC pathogenic for humans. Samples obtained were examined for STEC using PCR methods. STEC strains detected from 39 (10.8 %) milk, 2 (5 %) cheese. They confirmed that dairy products important reservoir of STEC pathogenic for human.

Vidal et al. (2005) designed a multiplex PCR for the detection of all categories of diarrhoea genic ***Escherichia coli***. This method proved to be specific and rapid in detecting virulence genes from Shigatoxin-producing (Stx 1), Stx (2), and eae), enteropathogenic (eae and bfp), enterotoxigenic (StII and Lt), enteroinvasive (Vir F and ipaH), entero-aggregative (aaFII), and diffuse adherent (daaE) ***Escherichia coli*** in stool samples.

3- MATERIAL AND METHODS

3.1.Material

3.1.1.Samples

A total of 60 rectal swabs were collected from calves and lambs at different localities of Kafr-Elsheikh Governorate. Also, 42 rectal swabs were collected from children (2 – 8 years old), and 112 samples were collected from different foods [60 raw milk, 33 Kariesh cheese and 19 meat samples] as shown in Table (1).

The samples were collected, labeled and transported with minimum of delay in an ice box (4 – 5 °C) to the laboratory of Department of Microbiology, Fac. of Vet. Med., Alex. Univ. during the period from March to December 2005.

Table (1): Samples collected from animals, human , foods.

Samples	No. of samples
1-Animals (Rectal swabs)	
a-Calves	30
b-Lambs	30
2-Foods	
a-Raw milk	60
b-Kariesh cheese	33
c-Frozen meat	19
3-Human (Rectal swabs)	42

3.1.2.Media for bacteriological examination:-

3.1.2.1.Media used for primary isolation:

1-Nutrient broth medium ***(Cruickshank et al., 1975):***

It was used for incubation of samples before their inoculation on solid media.

2-MacConkey's agar medium ***(Oxoid, 1987):***

It was used as a selective medium for isolation of members of family Enterobacteriaceae.

3-Blood agar medium ***(Oxoid, 1987):***

It was prepared by adding 5 – 10% defibrinated sheep blood to blood agar base. This medium was used to detect the haemolytic activity of bacteria.

3.1.2.2. Media used for characterization of the bacterial isolates:

1-1% peptone water broth ***(Cruickshank et al., 1975):***

This medium was used for detection of indole production.

2-Glucose phosphate broth ***(Cruickshank et al., 1975):***

It was used in methyl red and Voges-Proskauer (MR / VP) tests.

3-Simmon's Citrate agar medium ***(Koneman et al., 1988):***

This medium was used for citrate utilization test.

4-Stuart's urea broth ***(Koneman et al., 1988):***

It was used to detect urease activity of the isolated bacteria.

5-Semi-solid agar medium ***(Cruickshank et al., 1975):***

This medium was used for detection of bacterial motility and preservation of the bacterial isolates.

3.1.3.Solutions and reagents:

1-Potassium hydroxide (3%) (KOH) ***(Quinn et al., 1994):***

It was used to differentiate between Gram-positive and Gram – negative microorganisms.

2-Gram's stain ***(Quinn et al., 1994):***

It was used for staining films from bacterial isolates for morphological characters and staining reaction.

3-Tetramethyle-P-Phenylendiamine dihydrochloride (1.0%). (Oxidase reagents). ***Koneman et al., 1988):*** It was used for oxidase test.

4-Hydrogen peroxide (3%) (H2O2) ***(Koneman et al., 1988):*** This solution was used for catalase test.

5-Kovac's reagent ***(Koneman et al., 1988):***

It was used for detection of indole production.

6-Methyl red indicator ***(Koneman et al., 1988):***

It was use as a pH indicator for methyl red test.

7-Voges-Proskauer reagents ***(Koneman et al., 1988):*** 5 % α naphthol and 40 % KOH.

8-Reagents and chemicals used for polymerase chain reaction (PCR):

a-Oligonucleotide primers:

-The primers were selected to amplify ***E. coli*** shiga – like toxin SLT II gene ***(Fractamico et al., 2000)*** :-

i-SLTII-F GTTTTT CTT CGG TAT CCT ATT CC

ii-SLTII-R GAT GCA TCT CTG GTC ATT GTA TTAC

These primers were synthesized and supplied by Gibco BRL life Technologies Inc.

b-Ready –To-GO™ PCR Beads (Amersham pharmacia Biotech. Inc.):

These beads contain the following ingredients: Taq DNA polymerase,

- 10 x reaction Buffer (Kc 1500 mM and Tris-HCl 100 mM pH 9.0),
- 25 mM MgCl2 and dNTP mix (10 mM of each dNTP).

c-Agarose HGT (P):

It was purchased from Nacali Tesque, Inc, Kyoto, Japan. It was used for preparation of 2.0% agarose gel. The gel was used for detection of PCR products by electrophoresis.

d-Mineral oil (Sigma):

It was used to cover the PCR mixture to prevent evaporation during thermocycling.

e-Absolute ethanol:

It was purchased from El-Nasr Pharmacological, Co., Egypt. It was used for precipitation of DNA.

f-Tris-HCl (1 M) ***(Sambrook et al., 1989):***

It was prepared by dissolving 121.1 g of Tris base in 800 ml of distilled water. The pH was adjusted to the desired value by adding concentrated HCl. The buffer volume was completed to one liter with distilled water and then sterilized by autoclaving. It was used for preparation of Tris-EDTA buffer solution (TE).

g-EDTA (0.5 M – pH 8.0) ***(Sambrook et al., 1989):***

186.1 g of Di-sodium EDTA was dissolved in 800 ml of distilled water. The pH was adjusted to 8.0 with 10 N Sodium hydroxide. The solution was completed to one liter and

autoclaved. It was used for preparation of Tris-EDTA (TE) and Tris-acetate EDTA (TAE). buffers.

h-Tris-EDTA buffer (TE) (pH 8.0) ***(Sambrook et al., 1989)***:

1 M Tris – HCl	1.0 ml
400 mM EDTA (pH)	0.2 ml
Double distilled water up to	100 ml

It was sterilized by outoclaving and used for dissolving the DNA pellet after ethanol precipitation.

i-Tris-acetate EDTA (TAE) buffer ***(Sambrook et al., 1989):***

50 x TAE buffer : It was prepared as follows:

Tris base	242.0 g
Glacial acetic acid	57.1 ml
0.5 M EDTA (pH 8.0)	100 ml
Distilled water up to	1000 ml

It was sterilized by outoclaving and stored at room temperature. It was used for preparation of agarose gel and running buffar for electrophoresis.

j-Gel-loading buffer (6 x buffer) ***Sambrook et al., 1989)***:

0.25%	bromophenol blue.
40% (w/v)	sucrose in water.

This buffer was stored at 4 °C. This gel-loading buffer serves three purposes:

i-It increases the density of the samples, ensuring that the nucleic acid (PCR product) drops evenly into the gel well;

ii-It adds colour to the sample, theraby simplifying the loading process;

iii-It contains a dye that, in an electric field, moves, towards the anods at a predictable rate.

k-Ethidium bromide:

It was obtained from Amersham pharmacia Biotech. Inc. It was used at a concentration of 0.5 µg/ml for staining the DNA in the agarose gel

l-DNA marker:

100 Base pair ladder (1 µg/ml) was purchased from Amersham Pharmacia Biotech. Inc. It was used as a DNA molecular weight marker.

3.2.Methods

3.2.1. Collection of samples:

Rectal swabs were taken from diarrhoeic animals (calves, lambs) and children by means of sterile cotton swabs ***(Boyd et al., 1974)***. Also the foods samples (milk, cheese, meat) were collected. The collected samples were transferred in an ice box to the laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, Alexandria University. The samples were subjected to bacteriological examination as soon as possible.

3.2.2.Bacteriological examination:

3.2.2.I-Cultivation of the samples for isolation of *E. coli:*

Rectal swabs either from animals or children and food samples were inoculated into nutrient broth and incubated aerobically at 37 °C for 18 – 24 hours. A loopful of nutrient broth was streaked on to MacConkey's agar medium. The inoculated medium was incubated aerobically at 37 °C for 24 – 48 hours, then examined for bacterial growth.

Suspected colonies were picked up and purified. Purified colonies were streaked onto nutrient agar slopes. Stock cultures were preserved into semi-solid nutrient agar medium.

3.2.2.2.Identification of the isolates:

The bacterial isolates were characterized according to their morphological, cultural, and biochemical characteristics as well as their motility as follows:

1-Morphological characteristics:

Films were prepared from fresh cultures, stained with Gram's stain and examined microscopically for the morphological characteristics of the isolate.

2-Cultural characteristics:

The colonal morphology on Maconkey's agar and sorbitol maconkey's agar media were studied.

3-Detection of motility:

The isolates were inoculated into tubes containing semi-solid nutrient agar medium and incubated for 24 hours at 37 °C. Inoculated tubes were examined for detecting motility of the inoculated isolates, then preserved in the refrigerator at 4 °C.

3.2.2.3.Biochemical characterization:

Bacterial isolates were identified according to ***Edwards and Ewing (1972); Finegold and Martin (1982); Koneman et al. (1993) and Quinn et al. (2002)*** as follows:

1-Catalase test:-

This test was used to differentiate between bacteria that produce catalase enzyme from that which are non-catalase producer bacteria.

2-Oxidase test (Cytochrome oxidase test):-

It was used to determine the presence of oxidase enzyme which was produced by many Gram negative bacteria other than members of family Enterobacteriaceae. In positive results, the colonies turned to purple in colour then deep violet to black.

3-Indole test:-

To 48 hours peptone water culture, 5 drops of Kovac's reagent were added down the inner wall of the tube. The positive reaction was characterized by development of a bright fuchsia red colour at the interface of the reagent and the broth within seconds.

4-Methyl red test.

Methyl red is a pH indicator with a range between 6.0 (yellow) and 4.4 (red). The pH at which methyl red detects acid is considerably lower than the pH for other indicators used in bacteriologic culture media. Thus, in order to produce a colour change, the test organism must produce large quantities of acid from the carbohydrate substrate being used.

5-Vogas-Proskauer test.

The test depends on break down of glucose into intermediate product (acetyl methyl carbinol) (AMC) which is detected by addition of 5 % α-naphthol (in absolute ethyl alcohol) followed by addition of 40 % potassium hydroxide KOH (in Distilled water).

6-Citrate utilization test:

The test depends on the ability of tested bacteria to use citrate as a sole source of carbon. The tested bacteria were cultured on medium containing sodium citrate and ammonium salts with production of ammonia leading to alkalinization of medium for conversion of ammonia to ammonium hydroxide. A positiove test is development of a deep blue colour within 24 to 48 hrs indicating that the test organism has been able to utilize the citrate contained in medium, with production of alkaline products.

7-Urease test.

Urea is adiamide of carbonic acid with the formula

$$NH_2\text{-}\overset{\overset{O}{\|}}{C}\text{-}NH_2$$

All amides are easily hydrolyized with the release of Ammonia and carbon dioxide.

Urease is an enzyme possessed by many species of microorganisms that can hydrolyse urea which result in alkalinization and increase in the pH of medium that due to Ammonia reacts in solution to form ammonium carbonate.

3.2.2.4.Detection of haemolytic property of *E. coli* isolates:

The isolated colonies were streaked onto 5% sheep blood agar plates and incubated for 24 hours at 37 °C and examined for their haemolytic activity.

3.2.3.Polymerase Chain reaction:- (PCR):-

a- Bacterial template DNA:

DNA template was extracted from *E. coli* cells according to ***Nishikawa et al. (1988).*** *E. coli* isolates were grown in LB broth overnight at 37 °C. 100 µL of broth culture were centrifuged and the pellet was resuspended in distilled water . The genomic DNA was extracted by boiling of the suspension for 10 minutes and the supernatent was used as a template for polymerase chain reaction.

b- Oligonucleitide primers set SLT-II:

Primers were dissolved in nuclease-free water to obtain 50 – 100 mol concentration. 5 µl of the two primers were used in PCR mixture ***(Fratemico et al., 2000)***. The melting temperature (TM) of the upstream primer was 64 and for down stream was 70 as given by manufacture.

*The melting temperature ™ of each oligonucleotide using the formula Tm = 4 (G + C) + 2 (A + T), where G, C, A and T indicate the number of corresponding nucleotides in the oligonucleotide.

c-Programming the thermal cycler:

(ii) Programming the thermal cycler for detection of SLT-II gene: **(Fratamico et al., 2000):**

The thermal cycler was programmed as follows:

(i) One cycle for 2 minutes at 94 °C to denature the template DNA followed by (ii) 35 cycles of denaturation, annealing and extension at 94 °C for 20 seconds, 57 °C for 1 minutes and 72 °C for 1 minutes. The 35 cycles were followed by a final cycle of extension at 72 °C for 10 minutes to ensure that the entire PCR product is double strand DNA as shown in Table .

Table (2): Setting up the thermal cycler for SLT-II gene:

No. of cycles	°C	Time	Target
1 cycle	94	2 min	DNA template denaturation
35 cycles of	a) 94	20 sec.	Denaturation
	b) 57	1 min.	Primer annealing
	c) 72	1 min	Extension
1 cycle	72	10 min	Final extension
1 cycle	4	∞	preservation

-Polymerase chain reaction protocol ***(Ojeniyi et al., 1994)***

The reaction was conducted in a total volume of 25 µL in 0.5 ml microfuge tube Ready – to - Go™ PCR beads commercially supplied from AmerSham Pharmacia Biotech. Inc. The beads contain all the components of PCR except primers and DNA template. The reaction was carried by addition of 12 µ of nuclease-free water, five µl of each primer, and three µL of the template DNA to the beads in the microfuge tube. The reaction mixture was overlaid with 40 µL of nuclease free mineral oil to prevent evaporation. After that the tubes were placed in the thermal cycler previously programmed and the reaction started. At the end of cycling the tubes were stored at – 20 °C until being used.

d-Detection of PCR products *(Sambrook et al., 1989):*

Once the polymerase chain reaction completed, the next step was to detect the presence of specific products. Agarose gel electrophoresis was used for detecting PCR products using Hoefer Minnie Submarine agarose gel unit, Model HE-33. The procedure was carried out as follows:

-1.0 g of agarose was dissolved in 100 ml of 1 X TAE buffer in a boiling water bath.

-The melted agarose was cooled to 60 °C.

-The comb was put 0.5 – 1.0 mm above the plastic tray so that complete wells are formed when agarose was added.

-The melted agarose was poured into the mould. The thickness of the gel should be between 3 – 5 mm.

-After solidification of the gel, the comb was carefully removed. The gel with plastic support was put in the electrophoresis tank.

-A sufficient amount of 1 X TAE was added to electrophoresis tank to cover the gel by about 1- mm.

-5 µL of the PCR product were mixed with 1 µL of 6 X gel-loading buffer. The mixture was slowly loaded into the wells of the submerged gel using disposable micropipette. One well of the gel was loaded with 2 µL of 100 bp DNA ladder.

-The lid of the electrophoresis tank was closed and the electrical leads were connected.

-A constant voltage of 100 Volt was applied and the gel was run until the bromophenol blue has migrated half way down the gel. Usually one hour was enough to complete this.

-The electric current was switched off. The leads and lid were removed from the gel tank.

-The gel was stained with ethidium bromide in TAE buffer (0.5 µg/ml) for 30 – 45 minutes at room temperature.

-The stained gel was visualized by UV-transillumination (UVP ultraviolet products, TLW-20 transillumination, Amersham Pharmacisa Biotech. Inc.) and examined for presence of predictable bands.

-The size of the bands was compared with the bands of the DNA molecular marker. The presence of 244 bp DNA bands was regarded as a positive result for the existence of STa gene.

-Finally the agarose was photographed.

4-RESULTS

4.1.Incidence of *E. coli* isolated from diarrhoeic animals

A total of 60 collected rectal swabs (30 from calves and 30 from lambs) were examined bacteriologically. Identified ***E. coli*** was isolated from 10 calves (33.3%) and 8 lambs (26.7%), respectively as shown in Table (3).

Table (3): Incidence of ***E. coli*** isolated from diarrhoeic animals.

Animal species	No. of tested samples	No. of isolated ***E. coli***	Rate of isolation (%)
Calves	30	10	33.3
Lambs	30	8	26.7
Total	60	18	30

4.2.Incidence of *E. coli* isolated from human

A total of 42 rectal swabs from diarrhoeic children were collected and examined bacteriologically. Identified ***E. coli*** was isolated from 10 children (23.8%) as shown in table (4).

Table (4): Incidence of ***E. coli*** isolated from human.

Samples	No. of tested samples	No. of isolated ***E. coli***	Rate of isolation (%)
Diarrhoeic children	42	10	23.8 %

4.3.Incidence of *E. coli* isolated from foods

A total of 112 samples from different types of food [19 frozen meat , 60 raw milk and 33 Kariesh cheese samples] were collected. Identified ***E. coli*** was isolated from 10 milk (16.7 %), 7 meat (36.8%) and 9 Kariesh cheese (27.3 %), as shown in Table (5).

Table (5): Incidence of ***E. coli*** isolated from foods.

Type of food	No. of tested samples	No. of isolated ***E. coli***	Rate of isolation (%)
Raw milk	60	10	16.7 %
Frozen meat	19	7	36.8 %
Kariesh cheese	33	9	27.3 %
Total	112	26	23.2 %

Table (6): Incidence of ***E. coli*** isolated from animals, human and foods.

Samples	No. of tested samples	No. of isolated ***E. coli***	Rate of isolation (%)
Animals	60	18	30 %
Children	42	10	23.8 %
Foods	112	26	23.2 %
Total	214	54	25.20 %

4-4. Results of haemolytic activity of *E. coli* isolates:-

A total of identified 54 ***E. coli*** isolates were cultivated on blood agar medium. 5 isolates (9.25 %) showed α-haemolysin phenotype and 49 (90.75 %) showed no haemolysis as shown in Table (7).

Table (7): Results of haemolytic activity of identified ***E. coli*** isolates.

Total ***E. coli*** isolates	Haemolytic activity of E, coli isolates			
	α-haemolysis		No haemolysis	
	No.	Rate (%)	No.	Rate (%)
54	5	9.25	49	90.75

The five isolates which showed α-haemolysin phenotype were from lambs, human and milk [2 isolates from lambs (25 %), 1 isolates from human (10 %) and 2 isolates from milk (20 %)] as shown in table (8).

Table (8): Alpha-haemolysin of different isolates.

Samples	Samples	α- haemolysin	
		No.	Rate (%)
Lambs	8	2	25
Raw milk	10	2	20
Human	10	1	10
Total	28	5	17.9

4-5.Results of polymerase chain reaction for detection of *E. coli* SLT-II gene:

Ten isolates were tested for their virulence by detection of Shiga-toxin gene using Polymerase Chain Reaction. The isolates, which subjected to PCR were 3 isolates from calves, 2 isolates from lambs, 2 isolates from human, 1 isolate from frozen meat, 1 isolate from Kariesh cheese and 1 isolate from raw milk.

Table (9): Results of detection of Shiga-toxin gene of isolated ***E. coli*** by PCR.

Isolates	No. of examined isolates	Positive isolates		Negative isolates	
		No	%	No	%
Calves	3	3	100 %	0	0
Lambs	2	2	100 %	0	0
Human	2	2	100 %	0	0
Frozen meat	1	1	100 %	0	0
Kariesh cheese	1	1	100 %	0	0
Raw milk	1	1	100 %	0	0
Total	10	10	100 %	0	0

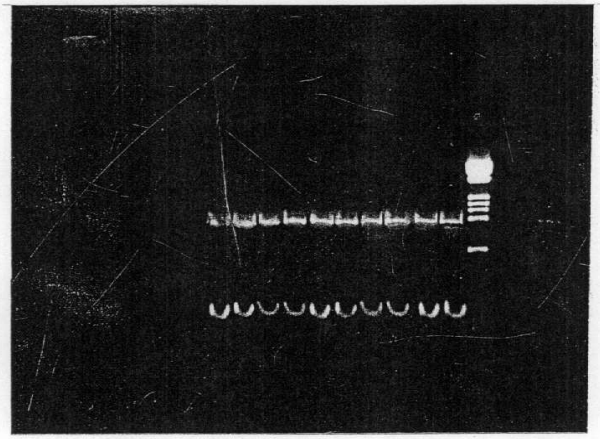

Fig. (1): Electrophoretic analysis of PCR amplified DNA of SLT-II.

5-Discussion

The native habitat for ***Escherichia coli*** is the enteric tract of man and animals. Its presence in foods generally indicates direct or indirect pollution of faecal origin ***(ICMSF, 1978)***. Foods of animal origin, may be contaminated with ***Escherichia coli*** from food handlers, food utensils, air, soil and water as well as under incomplete hygienic circumstances during manufacturing, packing , and marketing of these products ***(Frazier and Westhoff, 1978).***

Many investigators reported the isolation of ***E. coli*** from different types of meat ***(Shooter et al.;, 1970; Shooter et al., 1971; Cox and Mercuri , 1978 and Hassan, 1986).***

The presence of ***Escherichia coli*** as enteropathogens in meat and its products praides an evidence of contamination of faecal or water origin and reflects the unsatisfactory hygienic condition during manufacturing and handling of these products by human carriers ***(Mehlman et al., 1976 and Niazi and Refai, 1988).***

Freeman (1960) mentioned that ***Escherichia coli***, Coliform and Enterococci are considered as indicators for the sanitary quality of foods, and their presence in great numbers can easily rise to public health hazards.

Miskiman et al. (1976) found that ***Escherichia coli*** count was suitable as an indicator of inferior microbiological quality of foods, but of assure safety of food products , specific pathogen testing is necessary.

Hefnawy (1980) mentioned that using heat with additives to meat products proved to be beneficial as compared with meat untreated with additives in reducing the bacterial load of the products.

Hock (1969) found that the presence of ***E. coli*** in meat and its products was an indication of recent faecal contamination, the presence of Coliforms generally represent low hygienic conditions.

Mehlman et al. (1976) recorded that about 2 % of food poisoning cases along years (1969 – 1972) was attributed to enteropathogenic ***Escherichia coli*** (EEC).

Stiles and Lai-King (1981) reported that source of Enterobacteriaceae on meat was mainly from meat samples and work surface. They added that ***Escherichia coli*** biotype I was detected at all stages of meat handling and recognized as an indicator of direct faecal contamination of meat.

Several identifiable strains of ***Escherichia coli*** have been known for many years to cause infantile diarrhoea ***(Neter et al., 1951; Taylor and Charter, 1952).***

In recent years it has become increasingly evident that, ***Escherichia coli*** strains also produce illness to a significant degree in adults ***(Sakazaki et al., 1974 and Anon, 1978).***

Smith et al. (2004) cited that risk factors among children included caring for an ill calf and getting visible manure on their hands. Always washing hands with soap after touching a calf and washing hands before going home were protective. They also cited that calves were the reservoir of multiple enteric pathogens for children. Health caser providers should consider numerous zoonotic pathogens in patients presenting with gastroenteritis after contact with cattle.

Public health officials should help venne operators prospectively implement published guidelines to prevent zoonotic disease transmission.

PCR was a valuable and sensitive method for determining the virulence factors of ***E. coli*** strains and seemed to give good results in epidemiological investigation of diarrheogenic ***E. coli*** ***(Osek et al., 1999),*** with all these points in view, the objective of this study was to determine the prevalence rate and virulence characters associated with ***E. coli*** isolates.

Bacteriological examination of 214 samples collected from animals (60 samples), children (42 samples), and foods (112 samples, 60 from milk, 19 from meat and 33 from cheese), revealed that, ***E. coli*** was isolated in an incidence of 30 %, 23.8%, 16.7 %, 36.8 %, 27.3 %, respectively. As shown in Table (6) several studies have been showed differences in isolation rate of ***E. coli.***

Incidence of ***E. coli*** isolated from animals in this study was 30 % similar to the results obtained by ***Amrousi et al. (1972)*** (30 %) and ***Mona (1995)*** (33.33 %). Another studies showed results lower and higher than obtained by this study such as ***Morris et al. (1987)*** (12.1 %) , ***Viring et al. (1993)*** (11.5 %) , ***DelaFunte et al. (1998)*** (11.9 %) , ***Manaa et al. (1993)*** (80 %) , ***Steiner (1997)*** (42 %) , ***Perez et al. (1998)*** (94 %), ***Haggag and Khaliel (2002)*** (82 %) , ***Entsar (2004)*** (81.25 %).

The differences in isolation rates of ***E. coli*** may be attributed to environmental and managemental conditions (build up in calf house, the farm dirts and the adverse climatic changes) as reported by ***Acres, (1985), Hinton et al. (1994) and Draz et al. (1999).***

The incidence of ***E. coli*** isolated from children in this study was 23.8%. This result is nearly similar to that obtained by ***Addye et al. (2004)*** (14.8%) , ***Wierzba et al. (2006)*** (14 %) and ***Koehler et al. (2006)*** (32.7 %). Other researchers obtained results higher than that obtained by this study such as ***Asinobi et al. (2003)*** (64.9 %), ***Berkely et al. (2005)*** (70 %) , and ***Potgieter et al. (2005)*** (70 %).

The differences in isolation rates of ***E. coli*** could be attributed to personnel hygiene, environmental and managemental conditions around children, and also the difference in methods of isolation and identification of ***Escherichia coli***.

The incidence of ***E. coli*** isolated from meat in this study was 36.8%. This result nearly is similar to that obtained by ***Duitschaever et al. (1973)*** (39.8%) , and ***Faisal (1993)*** (32 %). However, other researchers obtained results lower than that obtained by this study such as ***Roushdy (1971)*** (24 %) and ***Youseef et al. (1984)*** (10 %). ***Mousa and Yassien (1987)*** (23 %), ***El-Mossalami et al (1988)*** (28.9%), ***Darwish et al. (1986)*** (20 %) and ***Zhao et al. (2001)*** (19%).

Other researchers obtained results higher than our result such as ***Iman (1989)*** (84 %) and ***Eman (1990)*** (48 %).

The incidence of ***E. coli*** isolated from cheese in this study was 27.3%. This result nearly is similar to that obtained by ***Shelaih (1979)*** (34.92 %) and ***Mohamed (1979)*** (34.27 %). Another studies showed lower results than that obtained in this study such as ***Fantasia et al. (1975)*** (10 %), ***Moustafa et al. (1975)*** (21 %) and ***Nagah (1984)*** (22 %) . Also there are several studies showed results higher than that obtained in this study such as ***El-Bassiony (1977)*** (67 %), ***Hassan (1983)*** (81 %), ***Eman (1986)*** (75 %), ***Ahmed et al. (1988 b)*** (84 %) and ***Amal (1990)*** (45.5 %).

The incidence of ***E. coli*** isolated from milk samples in this study was 16.7 %. This is nearly similar to that obtained by ***Maida et al. (1970)*** (11.2 %) , ***Singh et al. (1970)*** (16 %), ***Miltere et al. (1973)*** (25 %), ***Pandey and Mandal (1980)*** (10 %), ***Ergull (1982)*** (10.8 %), ***Nagah (1984)*** (24 %), and ***O' Ferrall-Berndt (2003)*** (17 %).

Another studies showed results higher than that obtained by this study such as ***Bogdanowicz and NockIewicz (1973)*** (98.34%), ***Saudi (1978)*** (36 %), **Adel (1978)** (36 %), ***Adel (1978)*** (36 %), ***Moustafa (1978)*** (73.33 %), ***Hassan (1983)*** (82.9 %) and ***Emam (1986)*** (66.6 %).

The virulence of ***E. coli*** was multifactoria and contain properties were associated primarily with virulent strains one of these was the ability to produce a haemolysin

(Cavalieri et al., 1984). The α-haemolysin was behind to be a virulence factor ***(Bohach and Snyder, 1985).***

The contribution of haemolysin production to virulence of ***E. coli*** isolates was attempted in this work using 5 % sheep blood agar plates, α-haemolysin was produced by 25 % (2/8) of ***E. coli*** isolates from lambs, 20 % (2 / 10) from raw milk, 10 % (1 / 10) from children, α-haemolysin ***E. coli*** isolates produced by 9.25 % from total isolates.

This result disagree with the result obtained by ***(Samer, 2001)*** who found that only 4.91 % (9 / 140) of ***E. coli*** isolates from diarrhoeic animals were α-haemolysin producing ***E. coli***, also the

The result obtained by ***(Gaber, 2004)*** who found only 10.2 % (5 / 49) of ***E. coli*** isolates from calves were α-haemolysin, and the result obtained by ***(Ibrahim, 1995)*** was 2.8 % of ***E. coli*** isolates from calves showed α-haemolysin.

El-Gannam and Sidorv (1986) reported 77.3 % (34 / 44) of ***E. coli*** strains isolated from animals were haemolytic, this result higher than the result obtained by the study.

In this study we used PCR as a genotypic method to detect gene encoding for SLT-II production. The result of PCR amplification of SLT-II gene revealed 100 %. ***E. coli*** isolates under PCR test non-haemolytic, and we choose 10 isolates only, 3 isolates from calves, 2 from lambs, 2 from human, and isolate from (raw milk, frozen meat, Kariesh cheese)from the results obtained, non haemolytic ***E. coli*** isolates was positive for PCR.

This results disagree with ***(Gaber, 2004)*** who isolated SLT-II producing ***E. coli*** from animals with a percentage 21.4 % (3 / 14) by PCR. ***Ibrahim (1995)*** obtained 2.5 % of isolates from calves were SLT producing ***E. coli***.

Cavalieri et al. (1984) cited that the virulence of ***E. coli*** was multifactorial and certain properties were associated with virulent strains. STEC (Shiga-toxin ***Escherichia coli***) infection was attributed to consumption of dairy products that were contaminated with animal faeces, thus, dairy cattle are considered reservoirs of STEC and can impose a significant health risk to human ***(Hussein and Sakuma, 2005).*** Highly virulent STEC strains are being shed by diarrhoeic calves and from farms located in a high incidence area of human STEC infection ***(MerCado et al., 2004)***.

It has been estimated that the attack rate of STEC infection among children ranged from 0.1 % to 71 % ***(Griffen and Tauxe, 1991).*** In addition ***Pai et al. (1988); Ramotor et al. (1995); Buteau et al. (2000) and Loirat (2001)*** isolated STEC from children with diarrhea with a rate of 3.2 %, 0.6 %, 12 % and 85 %, respectively. The differences in the reported prevalence rates could be attributed to difference in contamination rates in the studied areas.

Also, STEC strains detected in 126 (2.5 %) of 5054 cases of children with diarrhea, or other gastro-intestinal alteration ***(Blanco et al., 2004).***

Polymerase chain reaction positive for Shiga-toxin producing ***E. coli*** of milk samples with 16.3 % and 23.8 % from tank milk ***(Muehlher et al., 2003),*** 32.4 % of calves and 4.5 % of raw milk detected by primary screening of PCR (Khan et al., 2005). Also., STEC strains detected from 39 (10.8 %) milk , 2 (5 %) cheese ***(Rey et al., 2005).***

The majority of STEC isolated from meat samples (70.5 %) harbored both Stx1 and Stx2 genes, while the others harbored either Stx1 or Stx2.

They confirmed that dairy products important reservoir of STEC for human ***(Rey et al., 2005)***. Also, cattle are major reservoir of STEC pathogenic to ***(Blanco et al., 2004).***

6-SUMMARY

Diarrhoea is a clinical entity causes serious economic losses as it may lead to high mortality, weight loss or even late growth in different animals and even in human. It is caused by a combination of many risk factors.

The most important being is certain strains of ***Escherichia coli*** that possessing virulent factors and also other members of ***Enterobacteriaceae***. These pathogens are responsible for great mortality and various morbidity changes and at the same time constitute a hazard to public health.

The aim of this study was, achieved through isolation of ***E. coli*** from animals, human and foods , as well as, detection of Shiga toxin producing ***E. coli*** by PCR.

This study was carried-out on a total of 60 rectal swabs that collected from calves and lambs at different localities of Kafr-Elsheikh Governorate. Also, 42 rectal swabs that were collected from children, and 112 samples collected from different foods [60 milk, 33 cheese and 19 meat samples]. The samples were collected, labeled and transported with minimum of delay in an ice box to the laboratory of Department of Microbiology, Fac. of Vet. Med., Alex. Univ. during the period from March to December 2005.

The bacteriological examination was made for characterization of the causative bacterial agents of diarrhoea either from rectal swabs of animals or humans and samples of food.

Characterization and identification of the bacterial isolates were made using morphological characteristics, cultural characteristics, detection of motility, biochemical characterization (Catalase test, oxidase test, indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Urease test)

Also, the Polymerase Chain Reactions tests was used for identification of the causative bacterial agents found in the collected samples.

The most important results that obtained from this study includes:-

1- From a total of 60 collected rectal swabs (30 from calves and 30 from lambs), the purified ***E. coli*** was isolated from 10 (33.3%) and 8 (26.7%), respectively.

2- From a total of 42 rectal swabs collected from diarrhoeic children. Identified ***E. coli*** was isolated from 10 (23.8%).

3- Also, from a total of 112 samples collected from [19 frozen meat , 60 raw milk and 33 Kariesh cheese samples] were collected. Identified ***E. coli*** was isolated from 10 raw milk (16.7 %), 7 raw milk (36.8%) and Kariesh cheese 9 (27.3 %).

4- From a total of identified ***E. coli*** isolates cultivated on blood agar medium, 5 isolates (9.25 %) showed α-haemolysin phenotype and 49 (90.75 %) showed no haemolysis.

5- The results revealed that there was 5 isolates showed α-haemolysin phenotype which isolated from lambs, human, milk [2 isolates from lambs (25 %), 1 isolates from human (10 %) and 2 isolates from milk (20 %)], also showed α-haemolysis phenotype .

6- The results of polymerase chain reaction for ***E. coli*** SLT-II gene indicated that, the number of positive isolates constituted 100 %, the examined samples was 3 isolates from calves, 2 isolates from lambs, 2 isolates from human, one isolate from frozen meat, one isolate from Kariesh cheese, and one isolate from raw milk.

7-This study concluded that the ***E. coli*** can transmitted and isolated from different animals, human and food sources and causes severe economic and risk factors to the human and animals. Also, PCR is an ideal tool for identification and characterization of Shiga toxin producing ***E. coli.***

7- REFERENCES

Acres, S. D. (1985): Enterotoxigenic ***Escherichia coli*** infection in newborn calves. J. Dairy Sci., 68 (1): 229 – 256.

Adel, M. M. (1978): Microbiological studies on raw and pasteurized milk. M. V. Sc. Thesis. Food Hygiene. Cairo Univ.

Adetosoy, A. I. (1986): E. coli : O. K. H. Serotypes strains in Nigeria frim kids, lambs, calves and piglets with diarrhoea , Bull of Animal Health and production in Africa. 33, 227.

Addy, P. A. ; Antepim, G. and Frimpong, E. H. (2004): Prevalence of pathogenic ***Escherichia coli*** and parasites in infants with diarrhoea in Kumasi. East. Afri. Med. J.; 81 (7): 353 – 7.

Adwan, G. M. and Adwan, K. M. (2004): Isolation of Shiga-toxin ***Escherichia coli*** from meat in Palestine. In. J. Food. Microbiol.; 97 (1): 81 – 4.

Ahmed, S. A. (1980): Calf sources in Egyptian buffalo-cows. Egyptian German Seminar on the mortality of Newly-born calves. PP. 19 – 21.

Ahmed, S. A. (1980): Studies on acute gastrointestinal infection in Assuit Governorate. Ph. D. Thesis fac. Med. Assuit Univ.

Ahmed, A. A.; H. A. Abdel-Rahman and M. K. Moustafa. (1988a): Incidence of Enterobacteriaceae in some selected food stuffs. Assuit. Vet. Med. J. 20 (40): 104 – 108.

Ahmed, A. A.; S. H. Ahmed and M. K. Moustafa. (1988b): Occurrence of faecal coliforms and Enteropathogenic ***Escherichia coli*** (EEC) in Egyptian soft cheese. J. Food Prot., 51 (6): 442 – 444.

Alikaer, V. A. and Zaroza, V. G. (1973): New findings concerning the causal agents of Colibacteriosis in calves. Trudy Moskovskoi Veterinarni Akademii, 65: 98 – 100.

Al-Khayyat, A. .; Abd-Allah, I. S.; Zafer, S. A. W. and Habasha, F. G. (1977): Severe outbreaks of calf scours in Baghdad province. Egypt. Vet. Med. J., 25, 221.

Amal, A. A. (1990): Occurrence of coliforms and enteropathogenic ***Escherichia coli*** (EEC) in locally manufactured cheese. M. V. Sc. Thesis. Milk Hygiene. Assuit Univ.

Amrousi, S.; Nafei, E. K.; El-Rehawi, M. and Abdel-Motilib. (1972): Studies on enteritis in buffalo calves in Assiut. J. Egypt. Vet. Med. Ass., 31 (34): 219 – 225.

Anon, L. (1978): Foodborne disease bacteria. In International commission on microbiological specifications for foods, Microorganisms in foods, I. Their significane and enumerations, 2nd. Ed., P. 22. Toronto; University of Toronto Press.

Asinobi, A. O.; Fatunde, O. J., Brown, B. J.; Osinusi, K. and Fasina, N. A. (2003): Urinary tract infection in febrile children with sickle cell anaemia in Ibadan. Ann. Trap. Pediatr; 23 (2): 129 – 34.

Aslani, M. M. and Bouzari, S. (2003): An epidemiological study on verotoxin-producing ***Escherichia coli*** (VTEC) infection among population of Northern region of Iran. Eur. J. Epidemiol. 18 (4): 345 – 9.

Asma. O. A.; Abd-El-Wahed, Z. H.; Kohli, K. H. and El-Sheikh, A. R. (1996): Some studies on clinical, hematological and biochemical changes in diarrheic neonatal buffalo calves with reference to hygienic conditions. Assiut Vet. Med. J., 35 (69): 91 – 101.

Bastaureous, A. F.; Thabet, A. El-R.; Abdel Hafeiz, M. M.; Sayed, A. M. and Arafa, M. I. (2001): Studies of diarrhoea in lambs in Assuit governorate. I. Isolation and identification of causative bacterial and parasitic agents. Assuit-Vet. Med. J. 46 (91): 98 – 108.

Berkley, J. A.; Lowe, B. S.; Mwangi, I.; Williams, T. and Bauni, E. (2005): Bacteremia among children admitted to a rural hospital in Kenya. N. Engl. J. Med.; 352 (1): 39 – 47.

Blanco, J. E.; Blanco, M.; Alonso, M. P.; Mora, A. And Caira, M. A. (2004): Serotypes, virulence genes, and intimin types of Shiga-toxin (Verotoxin) producing E. coli. Isolates from human patients. J. Clin. Microbiol., 42 (1): 31 – 9.

Boetz, A. L. (1978): Pathogenic relationships of rotovirus, ***E. coli*** and other agents in mixed infections in calves. J. Am. Vet. Med. Assoc., 1735 (2): 577 – 583.

Bohach, G. A. and Snyder, I. S. (1985): Chemical and immunological analysis of the Campler structure of ***E. coli*** α-HLy. J. Bacteriol., 164 (3): 1071 – 1080.

Borczyk, A. A.; Lior, H. and Ciebin, B. (1987): False positive identification of ***E. coli*** O157 in foods. Int. J. Food Microbiology., 4: 347 – 349.

Bogadanozicz, A. and NockIewicz, A. (1973): studies on microflora of raw milk and soured cream. Rocznilki Panstwowego Zakladu Hygiene. 24 (6): 731. dairy Science. AST., 36, 364.

Bolton, F. J. (2003): Milk borne general outbreaks of infectious intestinal disease. Epidemiol. Infect., 130 (3): 461 – 8.

Booth, L. and Rowe, B. (1993): Possible occupational acquisition of ***Escherichia coli*** O157 infection . Lancet, 342: 1298 – 1299.

Boyd, J. W.; Walker, J. R. and Leyland, A. (1974): Neonatal diarrhoea in calves. Vet. Rec., 95 (14), 310 – 313.

Bridge, P. D. (1996): Classificatioon. In: Biological data analysis: mapproch, J. C. Fry, ed. IRL Press. Oxford, England, PP. 81 – 126.

Brooks, J. T.; Sowers, E. G.; Wells, J. G.; Greene, K. D.; Griffin, P. M.; Hoekstra, R. M. and Strockbine, N. A. (2005): Non shiga toxins-producing ***Escherichia coli*** infections in the United States. J. Infect. Dis. 192 (8): 1422 – 9.

Bulte, M.; Montenegro, M. A.; Helmuth, R.; Trumpf, T. and Renter, G. (1990): Identification of Verotoxin forming strains of ***Escherichia coli*** from healthy cattle and pigs by DNA-DNA colony hybridization colony hybridization assay. Berlinerund Munchener. Tierarztliche Wochenschrift, 103 (11): 308 – 384.

Buteau, CV.; Prolx, F.; Chaibou, M.; Raymond, A.; Clermont, M. J.; Mariscalco, M. M. and Seidman, E. (2000): Leulocytosis in children with ***Escherichia coli*** O157 : H7 enteritidis developing the haemolytic uraemic syndrome. Pediatri. Infect. Dis. J. 19 (7): 642 – 647.

Byomi, A.; Herbst, W. and Baljer, G. (1996): Some viral agents associated neonatal calf diarrhoea . Assuit. Vet. Med. J. 40 (80): 156 – 167.

Caprioli, A.; Flabo, V.; Roda, L. G.; Ruggeri, F. M. and Zona, C. (1983): Partial purification and characterization of an ***E. coli*** toxic factor that induces morphological cell alterations. Infect. Immun., 39: 1300 – 1306.

Caro, I.; Feranadez-Barata, V, M.; Alonso, A. and Garcia, M. R. (2006): Detection, occurrence and characterization of ***Escherichia coli*** from raw milk in Spain. J. Food Prot. 69 (4): 920 - 4.

Cavalieri, S. J.; Bohach, G. A. And Syndor, I. S. (1984): ***Escherichia coli*** α-haemolysin: Characteristics and probable role in the pathogenicity. Microbiol. Rev., 48 (4): 326 – 343.

Chapman, P. A. (2000): Sources of ***Escherichia coli*** O157 and O157 and experiences over the past 15 years in Sheffield, U. K. J. Appl. Microbiol., 885: 515 – 605.

Chapman, P. A.; Wright, D. J. and Siddons, C. A.. (1994): A comparison of immunologic separation and direct culture for the isolation of verocytotoxin producing ***Escherichia coli*** O157 from bovine faces. J. Med. Microbiol. 40 (6): 424 – 427.

Cook, B. C.; Edwards, S. M.; Gleeson, T. W. and Thomson, A. E. (1983): A comparison of minimature test kits with conventional tests for the confirmation of ***Escherichia coli*** isolated from dairy products. Australian Journal of Dairy technology, 38 (4): 157 – 159.

Cox, E. D. and Mercuri, C. L. (1978): Comparison of two Miniikits (Aptand R. B.) for identification of Enterobacteriaceae isolated from poultry and meat products. J. Food Protection, 41, No., 2: 107 – 110.

Cruickshank, R.; Duguid, J. P.; Marmian, B. P. and Swain, R. H. A. (1975): Medical Microbiol. Vol. 2, The practice of medical Microbiol 12th ed., Churchill Livingstone, Edinburgh, London.

Darwish, A.; Hamdy, M. and Nouman, T. M. (1986): Quality evaluation of market meat pastes. Vet. Med. J., 34, 1: 37 – 48.

Damodaran, S. and Sundararaj, A. (1974): A survey of calf mortality in Madros State. Ind. Vet. J., 51 (5): : 359 – 364.

De La Funte, R.; Garcia, A.; Ruiz-Santa-Quiteria, J. A.; Luzon, M.; Cid, D.; Garcia, S.; Orden, J. A. and Gomez-Bautista, M. (1998): Proportional morbidity rates of enteropathogens among diarrheic dairy calves in centeral Spain. Prev. Vet. Med., 36 (2): 145 – 152.

Deucn, M.; Tomescu, V, and Rosu, A. (1974): Serotypes of ***Escherichia coli*** isolated from milk and from some sources of milk contamination. Igiena, 23, 297. dairy Sci. Abst. 37, 268.

Doran, C. R. (1988): Haemolytic colitis and haemolytic uraemic syndrome caused by ***Escherichia coli*** in people consuming undercooked beef and unpasteurized milk. J. Am. Vet. Med. Ass., 193 (11): 1360 – 1361.

Doran, C. R. and Angrick, E. J. (1991): Serotype O157 : H7 ***Escherichia coli*** from bovine and meat sources. J. Clin. Microbiol., 29 (6): 1225 – 1231.

Doyle, M. P. and Schoeni, J. L. (1987): Isolation of ***Escherichia coli*** O157 : H7 from retail fresh meat and poultry. J. Appl. Eviron. Microbiology., 53 (10): 2394 – 2396.

Draz, A. A.; Samy, M. and Bauymi, A. (1999): Role of environment in occurrence of calf diarrhoea. Assiut Vet. Med. J. Vol. 40 No. 80. 156 – 167.

Duhamel, G. E.; Moxely, R. A.; Maddox, C. W. and and Erickson, E. D. (1992): Enteric of a goat with enterohaemorrhagic E. coli. J. Vet. Diagnosis. Invest., 4: 197 – 200.

Duitschaever, C. L.; Arnott, D. R. and Bullook, D. H. (1973): Bacteriological evaluation of raw refrigerated ground beef. J. Milk Food Technol., 36, 375 – 377.

Edelman, T. and Levine, M. M. (1983): Summary of a workshop on enteropathogenic Escherichia coli. J. Infect. Dis., 147: 1108 – 1118.

Edwards, P. R. and Ewing, W. H. (1972): Identification of Enterobacteriaceae, 4th Ed., Burgess Publ., Minneapolis. 430 – 434.

El-Bassiony, T. A. (1977): Incidence of pathogenic micro-organisms and viability of some food poisoning bacteria in Kariesh cheese during storage. Ph. D. Thesis Fac. Vet. Med. Assuit. Univ.

El-Ged, A.; El-Sayed, Z. M.; Khalid, A.; Abd-El-Gaber, G.; Abd El-Rahman, M. and El-Bardisy, M. M. (1994): Studies on the role of clostridial organism and other bacteria in calf diarrhoea with special reference to their susceptibility to some antibacterial agents agents, Assuit. Vet. Med. J. 30 (60): 194 – 207.

El-Gannam, M. L. and Sidorv, M. A. (1986): Toxic and pathogenic properties of Escherichia coli. Veterinariya. (Moscow), 22: 66 – 67.

El-Mosslami, S. M. S.; Zienab, M. N. and Mohamed, H. (1988): Sanitary status of meat in rural area of Kalyobia Governorate. Vet. Med. J., 36, 3: 385 – 400.

Emam, H. A. (1986) : Sanitary condition of milk, fermented milk, kariesh cheese and Butter manufactured in Assuit province. M. V. Sc. Thesis. Fac. Vet. Med. Assuit. Univ.

Eman, M. S. Z. (1990): ***Escherichia coli*** in meat products with special reference to enteropthogenic strains. Egypt. M. V. Sc., Thesis. Fac. Vet. Med. Cairo Univ.

Entsar, H. M. (2004): Some epidemiological studies of calf diarrhoea . M. V. Sc. Thesis. Animal Hygiene. Fac. Vet. Med. Alex. Univ.

Ergullu, E. (1982): Studies on G-ve bacteria in milk. XXI. International Dairy Congress, Vol. I., Book I, Moscow, USSR, Mir Publisher, 131-Dairy Sci. Abst., 45, 410.

Erlich, H. A.; Gelfand, D. and Sninsky, J. J. (1991): Recent advances in the polymerase Chain reaction. Science, 252: 1643 – 1651.

Faisal, A. A. (1993): Contamination of meat and meat products with human bacterial pathogens. M. V. Sc. Thesis Microbiology Dep. Fac. Vet. Med. Assuit Univ.

Fantasia, L. D.; Mestrandrea, L.; Schrade, J. P. and Yager, J. (1975): Detection and growth of enteropathogenic ***E. coli*** in soft ripened cheese. Appl. Microbiol., 29, 2: 179 – 185.

Farid, A.; Ibrahim, M. S. and Rafai, M. (1976): Studies on Colibacillosis in Egypt. II: Incidence of ***E. coli*** serogroups among live calves. Zb1. Vet. Med. B. 23; 44.

Farid, A.F.;Lotfi, Z. S. and Abdel-Malek, A. S. (1979): Some observations on biochemical behaviour of coliform cultures. 14th Arab, Vet. Med. Congress, Cairo. 125.

Feng, P.; Monday, S. R. (2000): Multiplex PCR for detection of trait and virulence factors in enterohaemorrhagic ***Escherichia coli*** serotypes. Mol. Cell probes. 14 (6): 333 – 7.

Finegold, S. M. and Martin, W. J. (1982): Diagnostic microbiology. 6th Ed., C. V. Mosby Co., St. Louis, Toronto, London.

Floyd, T. M. and Blakemore, C. F. (1954): Isolation of human enteric pathogens from ready-to eat meats from butchershops in Cairo, Egypt. J. Inf. Dis. 94: 30.

Foster, T. A.; Fowler, J. A. Meslc. Lodiges, and Warreno, C. (1977): A bacteriological survey of raw ground beef. Journal of Food Protection. Vol. 40, No. 11, 790 – 794.

Framtamico, P. M.; Bagi, K. L. and Pepe, T. (2000): Amultiplex polymerase chain reaction assay for rapid detection and identification of ***E. coli*** O157 : H7 in Foods and Bovine faces. Journal of Food protection. Vol. 68, No.8, 1032 – 1037.

Frankel, G.; Philips, A. D.; Rosenshine, I.; Dougan, G.; Kaper, J. B. and Knutton, S. (1998): Enteropathogenic and enterohaemorrhagic ***Escherichia coli***: More subversive elements. Molecular Microbiology. 30, 911 – 921.

Franzolin, M. R.; Alves, R. C.; Keller, R.; Gomes, T. A.; Beutin, L. and Barreto. (2005): Prevalence of diarrhoea genic ***Escherichia coli*** in children with diarrhoea in Salvador, Bahia, Brazil. Mem. Inst. Oswaldo. Cruz; 100 (4): 359 – 63.

Fratamico, P. M. and Strobaugh, T. P. (1998): Stimulaneous detection of salmonella Spp. and ***E. coli*** O157: H7 by multiplex PCR. J. Ind. Microbiol. Biotechnol. 21: 92 – 98.

Frazier, W. C. and Westhoff, D. C. (1978): Food microbiology. 3rd Ed., Tata McGraw Hill Publ. Comp. Lted, New Delhi.

Frazier, W. C. (1979): Food microbiology. 2nd Ed., New York Mc Graw Hill Inc.

Freeman, W. H. (1960): The science of meat and meat products. American meat institute foundation Reinhold Publishing Corporation, New York.

Fwasher, E. W. and Martienz, A. A. (1975): Studies of neonatal calf diarrhoea . I. Fluid balance in spontaneous enteric colibacillosis. Br. Vet. J., 131: 190 – 197.

Fwasher, E. W.; Martinez, A. A.; Trainin, Z. and Marion, R. (1975): Studies of neonatal calf diarrhoea . II- Serum and faecal immune globulin in enteric colibacillosis. Brit. Vet. J., 131: 402 – 407.

Gaber, S. (2004): Polymerase Chain Reaction and DNA electrophoretic pattern of certain types of Escherichia coli. Ph. D. Thesis. Dept. of Microbiol. Fac. Vet. Med., Alex. Univ.

Garber, L. P. S. J.; Wells, D. D.; Hancock, M. P.; Doyle, J.; Tuttle, J. A. Shere and T. Zhao. (1995): Risk factors for fecal shedding of ***Escherichia coli*** O157 : H7 in dairy calves. JAVM. 207 : 46 – 49.

Garcia, A.; Ruiz-Santa-Quiteria, J. A.; Orden, J. A.; Cid, D.; Sanz, R.; Gomez-Bautista, M. and de la Fuente, R. (2000): Rotavirus and concurrent infections with other enteropathogens in in neonatal diarrhoea of dairy calves in Spain. Comp.Immunol. Microbiol. Infection. Dis., 23 (230: 175 – 183.

Gillespie, I. A.; Adak, G. K.; O' Brien, S. J. and Bolton, F. J. (2003): Milk borne general outbreaks of infectious intestinal diseas. Epidemiol. Infect., 130 (3): 461 – 8.

Glantz, P. J.; Simpson, M.; Wilson, L. L. and Straley, E. T. (1972): ***E. coli*** O73 : H18 in ***Colibacillosis*** of calves. J. Vet. Med. Small. Anim. Clinic., 67: 427 – 429.

Glennan, M.; Fitzmaurice, J.; Duffy, G.; Sheridan, J. J.; Caroll, Maher, M. (2004): Application of real-time PCR and RT-PCR assays for the detection and quantitation of VT1 and VT2 toxin genes in ***E. coli*** O157 : H7. Mol. Cell – Probes; 18 (2): 123 - 32.

Gonzalez Garcia, E. A. (2002): Animal health and food borne pathogens: enterohaemorrahagic strains and other pathogenic Escherichia colivirotypes (EPEC, ETEC, EIEC, EHEC) Pol. J. Vet. Sci.; 5 (2): 103 – 15.

Griffen, P. W. and Tauxe, R. V. (1991): The epidemiology of infections caused by ***Escherichia coli*** O157 : H7, other enterohaemorrhagic ***E. coli*** and the associated haemolytic uraemic syndrome. Epidemiol. Reviews, 13: 60 – 98.

Haggag, Y. N. and Khaliel, S. A. (2002): Public health importance of certain bacteria isolated from calves and small ruminants. The 2nd Veterinary Congress, Fac. Vet. Med., Minufyia University, Egypt. 2 (1): 173 – 184.

Hall, G. A.; Reynolds, D. J.; Chanter, N.; Morgan, J. H.; Parson, K. P.; Debney, T. G.; Bland, A. P. and Bridger, T. C. (1985): Dysentry caused by ***E. coli*** (5102 – 9) in calves: Natural and Experimental Disease. Vet. Pathol., 22 (2): 156 – 163.

Hall, H. G.; Brown, D. E. and Lewis, K. H. (1967): Examination of market food for Coliform organisms. Appl. Microbiology. 15. 1062.

Hassan, A. I. I. (1986): Microbiological quality of ready to eat meat-meals in air catering. M. Vet. Sci. Thesis. Cairo Univ.

Hassan, M. G. E. (1983): Enterobacteriacea in milk and milk products. M. V. Sc. Thesis Food Hygiene. Vet. Med. Assuit Univ.

Hefnawy, Y. (1980): Studies on the sanitary improvement of processed meat. J. Food Prot., 33 (5): 2200-10.

Hock, R. (1969): Escherichia coli I as an indicator of fresh faecal contamination of food. Zentable-Baket. Para-Site Kee, I. Orig. , 211: 498. Dairy Sci. Abst. 32: 438.

Holland, R. E. (1990): Some infectious causes of diarrhoea in young farm animals. Clin. Microbiol. Rev., 3 (4): 345 – 375.

Hussein, H. S.; and Bollinger, L. M. (2005): Prevalence of Shigatoxin-producing ***E. coli*** in beef cattle. J. Food Prot., 68 (10): 2224 – 41.

Hussein, H. S. and Sakuma, T. (2005): Prevalence of Shigatoxin-producing ***Escherichia coli*** in dairy cattle and their products.J. Dairy. Sci.; 88 (2): 450 – 65.

Hinton, M.; Aleen, V. and Linton, A. H. (1994): The effect of the management of calves on the prevalence of antibiotic – resistant strains of ***Escherichia coli*** in their faeces. Letters in Appl. Microbiol., 19 (4): 197 – 200.

Higgns, J. A.; Belt, K. T.; Karns, J. S.; Russell-Anelli, J. and Shelton, D. R. (2005): Tir and Stx-positive ***E. coli*** in stream waters in a metropolitan area. Appl. Environm. MIcrobiol.; 71 (5): 2511 – 9.

Ibrahim, A. M. (1995): Sanitary studies on newly born calves: Ph. D. Thesis (Animal Hygiene), Fac. Vet. Med., Suez Canal Univ.

I.C.M.S.F. (1978): International Commission on Microbiological Specification for Food. Microorganisms in foods. Their significance and methods of environments. Vol. I. 2nd End. Univ. of Toronto Press. Toronto. Ontario, Canada.

Iman, A. A. F. (1989): Microbiological status of packed meat products and fish in Benisuef Governorate. Ph. D. Thesis, Fac. Vet. Med., Cairo Univ.

Isaacson, R. E.; Moon, H. W. and Schneider, R. A. (1978): Distribution and virulence of ***E. coli*** in the small intestine of calves with and without diarrhoea . Amer. J. Vet. Res., 39 (11): 1750 – 1755.

Janke, B. H.; Francis, D. H.; Collins, J. E.; Libal, M. C.; Zeman, D. H.; Johnson, D. D. and Neiger, R. D. (1990): ttaching and effacing ***Escherichia coli*** infection as a cause of diarrhoea in young calves. JAVMA, 196 (6): 897 – 911.

Jayappa, H. G.; Strayer, J. G. and Goodnow, R. A. (1984): Controlling ***Colibacillosis*** in neonatal calves. J. Vet. Med. Small animal Clinic., 79 (3): 388 – 394.

Jensen, R. and Swift, B. L. (1982): Diseases of sheep. 2nd Ed. Lea and Febiger Philadelphia, USA.

Jonsson, M.; Erikson, E.; Aspan, A.; Vagsholm, I.; Wahlstrom, H. and Engvall, A. (2001): Verotoxin producing ***E. coli*** O157 in two Swedish dairy herds. Svensk Veterin artidning, 53 (12): 627 – 630.

June, R. C.; Ferguson, W. W. and Twaifel, M. (1953): Experiments in feeding adult volunteers with ***E. coli*** O55 : B5, a coliform organism, associated with infants diarrhoea . Am. J. Hyg. 57: 222 – 236.

Karamali, M. A. (1989): Infection of verocytotoxin – producing ***Escherichia coli.*** Clin. Microbiol. Rev. 2, 15 – 38.

Karamali, M. A. (2004): Infection of by Shiga toxin-producing ***Escherichia coli:*** an overview . Mol. Biotechnol. 26 (2): 117 – 122.

Karamali, M. a.; Petric, M.; Lim, C.; Fleming, P. C. and Steel, B. T. (1983): Escherichia coli cytotoxin, haemolytic uraemic syndrome and haemorrhagic colitis. Lancet, 2: 1299 – 1300.

Karpman, D.; Papadopoulou, D. and Nilson, K. (2001): Platelet activation of Shiga toxin and circulatory factors as a pathogenic mechanism in the haemolytic uraemic syndrome. Blood 97: 3100 – 3108.

Khan, A.; Das, S. C.; Panja, P.; Datta, S.; Sikclar, A.; Yamasaki, S.; Takeda, Y.; Bhattacharya, S. K.; Nair, G. B. and Ramamurthy, T. (2005): Dairy farm investigation on Shiga-toxin producing ***Escherichia coli*** (STEC) in Kolkata, India with emphasis on molecular characterization. Epidemiol. Infect., 133 (4): 617-26.

Khera, S. S. and Danda, M. R. (1963): Gastroenteritis in young calves. II. Colibacillosis and other infections. Indian. J. Vet. Sci., 33, 12 – 15.

Koheler, K. M.; Lasky, T.; Fein, S. B.; Delong, S. M.; Hawkins, M. A. and Vugia, D. J. (2006): Population-based incidence of infection with selected bacterial enteric pathogens in children younger than five years of age. Pediatr. Inffect. Dis. J. 25 (2): 129 – 34.

Koneman, E. W.; Allen, S. D.; Dowell, V. R.; Janda, W. H.; Sommers, H. M. and Winn, W. C. (1988): Color Atlas of Diagnostic Microbiology. 3rd Ed., Lippincott Compony.

Koneman, E. W.; Allen, S. D.; Dowell, V. R.; Janda, W. H. and Sommers, H. M.. (1993): Colour Atlas and Textbook of Diagnostic Microbiology. 4rd Ed., J. B. Lippincott Co., New York.

Krogh, H. V. and Henriksen, S. A. (1985): Bovine cryptosporidiosis in Denmark-2-Cryptosporidium associated with calf diarrhoea . Nord-Net. Med., 37 (1): 42 – 47.

Kovaler, V.; Shuklin, V. and Violin, B. (1987): Effectiveness of pramycin in Colibacillosis of calves. Soviet Agr. Sc. , 12, 58.

Kwok, S.; Mach, D.; Mullis, K. B.; Poiez, B.; Ehrlich, C.; Blain, D.; Friedman-Kein, A. and Sninsky, J. (1987): Identification of human immunodeficiency virus sequences by using in vitro amplification and oligomer cleavage detection. J. Virol., 61: 1690 – 1694.

Lantz, P.; Hahn-Hagerdal, B. and Radstrom, P. (1994): Sample preparation methods and PCR-based detection of food pathogens. Trends Food Sci. Technol. 5: 384.

Leclercq, A.; Lambert, B.; Pierard, D. and Mahillon, J. (2001): Particular biochemical profiles for enterohaemorrhagic ***Escherichia coli*** O157 : H7 isolates on the ID32E system. J. Clin. Microbiol. 39 (3): 1161 – 1164.

Levin, M. M. (1987): ***Escherichia coli*** that causes diarrhoea enterotoxigenic, enteropathogenic, enteroinvasive, enterohaemorrhagic, enteroadherent. J. Infect. Dis. 155 (3): 377 – 389.

Litalien, C.; Proulx, F.; Mariscko, M. M. and Robitaille, P. (1999): Criculatory inflammatory cytokine levels in haemolytic uraemic syndrome. Pediator. Nephrol. 13: 840 – 845.

Little, C. L. and De Louvois, J. (1999): Health risks associated with unpasteurized goat's and ewe's milk on retail sale in England and Wales. APHLS Dairy products working Group study Epidemiol. Infect. 122 (3): 403 – 408.

Loirat, C. (2001): Post-diarroeal haemolytic uraemic syndrome. Clinical aspects. Arch. Pediat. 4: 776 – 784.

Mahmoud, A. R. (1993): Clinicopathological studies on some diseases of cattle calves and buffalo calves. Ph. D. Thesis (Clinical pathology), Fac. Vet. Med., Cairo University.

Maida, B.; Arca, S. U.; Maida, M. and Realacc, I. G. (1970): Bacterial typing of coli forms present in milk. Latt, 44, 179. dairy Sci., Abst. 33, 548.

Mainil, J. G.; Moseley, S. L.; Schneider, R. A.; Sutch, K.; Casey, T. A. and Moon, H. W. (1986): Hybridization of bovine ***E. coli*** isolates with gene probes for four enterotoxins (Stap, Stah, sTb, LT) and one adhesion factor (K99). Am. J. Vet. Res., 47: 1145 – 1148.

Manaa, A. M.; Sayed, A. M.; Thabet, A. El. R. and Abdel Fattah, A. M. (1993): Some microbial and blood biochemical studies on buffalo-calves suffering from enteritis. Assuit Vet. Med. J., 29 (58): 144.

Manzullo, A. and Esterez, D. J. (1965): Investigation for the presence of pathogenic ***E. coli*** in raw milk and cream in a zone of Buenos Aires. J. Renta. Asco. Med. Aregent. 79, 36. dairy Sci. Abst. 28, 205.

Marier, R.; Wels, J.; Swanson, R.; Collahan, S. and Mehlman, I. (1973): An outbreak of ***E. coli*** foodborne disease traced to imported French cheese, Lancet, 2: 1376.

Mayer, A.; Kalich, I. and Mehnert, B. (1964): Kalber Franheiten. Wiener Tierarztliche Monatsschrift. 51, 74 – 92.

Mckee, R.; Madden, R. H. and Gilmour, A. (2003): Occurrence of verocytotoxin – producing ***Escherichia coli*** in dairy and meat processing environments. J. Food Prot. 66 (9): 1576 – 80.

Mehlman, I. J.; M. Fish. Bien, S. L. Gorbach, A. C.; Sanders, E. L.; Eide, J. C. and Olsan, Jr. (1976): Pathogenicity of ***Escherichia coli*** recovered from food. J. Assoc. off. Anal. Chem. 59: 67 – 80.

Mellmann, A.; Bielaszewska, M.; Zimmerhackll, B.; Prager, Harmsen, D.; Tschope, H. and Karch, H. (2005): Enterohaemorrhagic ***Escherichia coli*** in human infection. In vivo evolution of a bacteril pathogen. Clin. Infectious. Dis., 41 (6): 785 – 92.

Mello, A. and Amaral, R. F. (1940): The coliform flora of minase cheese and alimentary infections. Study on the Escherichia aerobacter group. Rev. Industr. Anim. 3, 34. Dairy Sci. Abst. 4, 183.

Mercado, E. C.; Gioffre, A.; Rodriguez, S. M.; Cataldi, A.; Irinok, Cipolla, A. L. and Mendez, M. A. (2004): Non O157 Shiga-toxin producing ***Escherichia coli*** isolated from diarrhoeic calves in Argentina. J. Vet. Med. B. Infect. Dis. Vet. Public. Health. 51 (2): 82 – 8.

Milltere, J. B.; Chadle, A. and Rachid, M. S. B. (1973): Bacteriological quality of pasteurized milk produced by Tanis Centeral dairy plant. Archives de l' Institute Pasteur de Tunis, 50 (3), 231. dairy Sci., Abst. 38, 541.

Miskiman, D. K.; Berkowitz, K. A.; Solberg, M.; Riha, W. M.; Franke, J. R.; Buchanan, W. C. and Leary, V. O. (1976): Relationships between indicator organisms and specific pathogens in potentially hazardous foods. J. Food Sci. 41: 1001 – 1006.

Mohamed, A. S. (1979): Micfrobiological studies on Egyptian soft cheese. Ph. D. Thesis. Food Hygiene. Cairo University.

Mona, A. A. (1995): Studies on the bacterial causes of enteritis in buffalo-calves in Ismailia Governorate. M. V. Sc. Thesis. Fac. Vet. Med., Zagazig University.

Montenegro, M. A.; Bulte, M.; Helmuth, R.; Trumpf, T. and Renter, G. (1990): Identification of Verotoxin forming strains of ***Escherichia coli*** from healthy cattle and pigs by DNA-DNA colony hybridization colony hybridization assay. Berlinerund Munchener. Tierarztliche Wochenschrift, 103 (11): 308 – 384.

Moon, H. W.; McClarkin, A. W.; Isaacson, R. E.; Pohlenz, J.; Skartuedt, S. M.; Gillette, K. G. and Boetz, A. L. (1978): Pathogenic relationships of rotovirus, ***E. coli*** and other agents in mixed infections in calves. J. Am. Vet. Med. Assoc., 173, 5 (2): 577 – 583.

Morris, J. A.; Chanter, N. and Sherwood, D. (1987): Occurrence and properities of F4 (AH25) + ***Escherichia coli*** associated with diarrhoea in calves. Vet. Rec. 121 (9): 189 – 191.

Moursy, A. W. and Nasr, S. (1964): Studies on the sanitary condition of fesh kariesh cheese with special reference to the incidence of some food poisoning organism. J. Arab. Vet. Assoc. 24, 99.

Mousa, M. M. I. and Yassien, M. A. (1987): Enterobacteriaceae of slaughtered carcasses of sheep and goats with special references to Salmonella. Alex. J. Vet. Sci., Vol. 3. No. I.

Moustafa, M. K. (1978): Studies on the sanitary condition of market milk in Assuit. M. V. Sc. Thesis Vet. Med. Assuit Univ.

Moustafa, T. H.; Kamel, Y. Y.; Ahmed, A. A. and Ismail, A. A. (1975): Studies on the sanitary condition of market milk in Assuit province, Egypt. Part II: The incidence of potentially pathogenic micro-organism. Assuit. Vet. Med. J. Vol. II, No. 3 & 4, 183 – 191.

Muehlherr, J. E.; Zweifel, C.; Corti, S.; Blanco, J. E. And Stephan, R. (2003): Microbiological quality of raw goats and ewe's bulk tank milk in Switzerland. J. Dairy Science. 72; 86 (12): 3849 – 56.

Nagah, M. H. (1984): Incidence and public health importance of coliforms with special reference to enteropathogenic serotypes of ***E. coli*** in milk and some dairy products.

National Academy of Sciences. (NAS): (1985): Ntional Academy of Sciences; An evaluation of the role of microbiological critical for foods and food ingredients. National Academy Press. Washington D. C.

Nelson, S.; Clarke, R. C. and Karamali, M. A. (1998): Verocytotoxin-producing ***Escherichia coli*** (VTEC) infection. In palmer, S. R.; Lardsoulsby and simpson, DTH-zoonoses. Biology, Clinical practice and public health control. Oxford University Press. PP. 89 – 104.

Nenkov, M. (1974): Incidence of enteropathogenic ***E. coli*** in milk products. Veterinarnomedit Sinski Nauki, 11, (2), 79 – 84. Dairy Science Abst., 36, 579.

Neter, E. Webb.; Shumway, C. and Murdock, M. (1951): Study on etiology, epidemiology and antibiotic therapy of infantile diarrhoea , with a particular reference to certain serotypes of E. coli. Amer. J. Publ. Health. 41: 1490.

Niazi, M. Z. and Refai, M. (1988): Isolation of enteropathogenic and enterotoxigenic ***Escherichia coli*** from meat and cheese. Vet. Med. J. 1: 121 – 134.

O' Ferrall-Berndt, M. M. (2003): A comparison of selected public health criteria in milk from milk-shops and from a national distributor. J. S. Afr. Vet. Assoc., 74 (2): 35 – 40.

Ojeniyi, B.; Ahrens, P. and Meyling, A. (1994): Detection of fimbrial and toxin genes in ***E. coli*** and their prevalence in piglets with diarrhoea. The application of colony hybridization assay, polymerase Chain reaction and phenotypic assays. J. Vet. Med. (B)., 41: 49 – 59.

Oliveir, A. A.; Perdreira, P. A. and Almedia, M. F. (1989): Diseases of calves: I-Bacterial diarrhoea in sergipe state, Brazil, Arquivo, Brasileriro de Medicina Veterinaria. Enzootecina. 41 (3): 213.

Olsvik, O.; Popovic, T.; Skjerve, E. and Kofitsyo, S. (1994): Magnetic separation techniques in diagnostic microbiology. Clinical Microbiology Reviews. 7: 43 – 54.

Orden, J. A.; Ruiz-santa-Quiteria, J. A.; Garcia, S.; Cid, D. and De La Fuente, R. (2000): Quinolone resistance in ***Escherichia coli*** strains from diarrhoeic lambs in Spain. Vet. Rec. Nov. 11; 147 (20): 576 – 578.

Osek, J.; Gallien, P.; Truszcznski, M. and Protz, D. (1999): The used of polymerase chain reaction for determination of virulence factors of ***Escherichia coli*** strains isolated from pigs in Poland Comp. Immunol Microbiol Infect. Dis.; 22: 163 – 174.

Oxender, W. D., Newman, L. E. and Morrow, D. A. (1973): Factors influencing dairy calf mortality in Michigan. J. Am. Vet. Med. Assoc., 162 (6), 458 – 460.

Oxoid. (1987): Agents and main distributors, the manual. Sixth Edition.

Pai, C. H.; Ahmed, N.; Lior, H.; Johnson, W. M.; Sims, H. V. and Woods, D. E. (1988): Epidemiology of sporadic diarrhoea due to verotoxin-producing ***Escherichia coli***: A two year prospective study. J. Infect. Dis. 157: 1054 – 1057.

Pandey, J. N. and Mandal, L. N. (1980): Studies on the bacterial flora of raw milk of milk supply scheme, patna, in relation to the public health. Indian Veterinary J. 57, 2, 147.

Perez, E.; Kummeling, A.; Janssen, M. M.; Jimenez, C.; Alvardo, R.; Caballero, M.; Donado, P. and Dwinger, R. H. (1998): Infectious agents associated with diarrhoea of calves in the Canton of Tilaran, Costa Rica. Prev. Vet. Med., 33 (1 – 4): 195 – 205.

Potgieter, N.; Obicl, Bessong, P. O.; Igumbor, E. D.; Samie, A. and Nengobela, R. (2005): Bacterial contamination of Vhuswa- a local weaning food and stored drinking-water in impoverished house holds in the Venda region of South Africa. J. Health Popul. Nutr., 23 (2): 150 – 5.

Prohaszka, L. (1973): Factors predisposing calves from ***Escherichia coli*** enteritis. Annal. Med. Vet., 4 (1), 171 – 174.

Psassis, E. P.; Voyiazas, D. and Giannacoulas, D. (1994): Enteropathogenic strains of ***E. coli*** isolated from foods of animal origin. Deltion Euenikes Mikrobial. Etaireios. 19, 2: 92 – 102 (dairy Sci., Abstr., 38, 1).

Qadri, F.; Khan, A. L.; Faruque, A. S.; Begum, Y. A. and Sack, D. A. (2005): Enterotoxigenic ***Escherichia coli*** and Vibrio cholerae diarrhoea . Emerg. Infect. Dis., 11 (7): 1104 – 7.

Quinn, P. J.; carter, M. E.; Markey, B. K. and Carter, G. R. (1994): Clinical Veterinary Microbiology. Mosby. Yearbook Europe Limited.

Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. C. and Leonard, C. F. (2002): Vet. Microbiology and Microbial disease Textbook, MPG Books LTd, Bodmin, Cornwall P. 111.

Radostitis, O. M.; Blood, D. C. and Gay, C. C. (1994): Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses, 8th Ed., Bailliere-Tindall, London.

Ranatunga, P. (1974): Calf mortality in dairy farms in Srilanka. Ceylon Vet. J., 22 (11/2) 4 – 9.

Ramotar, K.; Henderson, E.; Szumski, R. and Louie, T. J. (1995): Impact of free verotoxin testing on epidemiology of diarrhea caused by vertotoxin-producing ***Escherichia coli***. J. Clinic. Microbiol. 33 (5): 1114 – 1120.

Reynolds, D. J.; Morgan, J. H.; Chanter, N.; Jones, P. W.; Bridger, J. C.; Debney, T. G. and Bunch, K. J. (1986): Microbiology of calves diarrhoea in Southern Britain. Vet. Rec., 119 (2): 34 – 39.

Rey, J.; Sanchez, S.; Blanco, J. E.; Hermosode, Mendoza, J. And Garcia, A. (2005): Prevalence, serotypes and virulence genes of Shiga-toxin-producing Escherichia coli isolated from ovine, caprine milk and other dairy products in Spain. Int. J. Food. Microbiol. 28 (4): 617 – 26.

Rodriguez, J. M. (1997): Detection of animal pathogens using the polymerase chain reaction (PCR). Vet. J. ; 153: 287 – 305.

Roushdy, S. (1971): Studies on market minced meat. M. V. Sc. Fac. Of Vet. Med. Cairo. Univ.

Rubin, S.; Cardeti, G.; Amiti, S.; Manna, G.; Ontorati, R.; Capriali, A. and Morabito, S. (1999): Verocytotoxin-producing Escherichia coliO157 in sheep milk. Vet. Rec. 9 (144) 2: 56.

Saiki, R. K.; Schart, S. J.; Faloona, A. F.; Mullis, K. B.; Erlich, H. A. and Arnheim, N. (1985): Enzymatic amplification of β-globin genomic sequence and resterection site analysis for diagnosis of sickle cell anaemia. Science, 230: 1350 – 1354.

Sakazaki, R.; Tamura, T. and Nakamura, A. (1974): Further studies on E. E. C. associated with diarrhoea l diseases in children and adults. Japan. J. Med. Sci. Biol. 27: 7.

Sakuma, M.; Urashima, M.; and Okabe, N. (2006): Verocytotoxin-producing Escherichia coli. Emerg. Infect. Dis., 12 (2): 323 – 5.

Sambrook, J.; Fritsh, E. F. and Maniatis, T. (1989): Molecular cloning. A laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Cold Spring, New York.

Samer, K. (2001): Laboratory diagnosis of some bacterial agents causing pneumonia and/or enteritis in ovines, caprines and bovines. Ph. D. Thesis. Dept. of Microbiol., Fac. Vet. Med., Alex. Univ.

Saudi, A. M. M. (1978): Microbiological studies on raw and pasteurized milk. M. V. Sc. Thesis Fac. Vet. Med. Cairo Univ.

Savarino, S. J.; Rao, M. R.; Abu-Elyazeed, R.; NaFicy, A. B.; Wierzba, T. F.; Abdel-Messih, I.; Shaheen, H.; Frenck, R. W. and Svennerholm, A. M. (2003): High disease burden of diarrhoea due to enterotoxigenic ***E. coli*** among rural Egyptin infants and young children. J. Clin. Microbiol; 41 (10): 4862 – 4.

Schmidt, H.; Beutin, L. and Karch, H. (1995): Molecular analysis of the plasmid-encoded hemolysin of ***Escherichia coli*** O157 : H7 strain EDL 933. Infection and immunity 63, 1055 – 1061.

Schoning, P. and Sagartz, J. (1995): Identifying gross pathologic changes in young lambs. Vet. Med., 80 (12): 95 – 100.

Shelaih, M. A. (1979): Microbiological studies on Egyptian soft cheese. Ph. D. Thesis, Milk Hygiene, Fac. Vet. Med., Cairo Univ.

Shooter, R. A.; Cooe, E. M.; Rousseau, S. A. and Breaden, A. L. (1970): Animal serotypes of ***Escherichia coli*** in the food of hospital patients. Lancet. 2: 226.

Shooter, R. A.; Faiers, M. C.; Cooke, E. M.; Breaden, A. L. and O' Farrel, S. M. (1971): Isolation of Escherichia, Pseudomonas aeruginosa and Klebsiella from food in hospitals Canteen and Schools. Lancet, 2: 390.

Singh, R. S.; Ranganathan, B.and Laxminarayana, H. (1970): Incidence of pathogenic ***E. coli*** in milk and milk products. XVIII. Dairy Congr. IE: 148. Dairy Scien. Abst., 32, 720.

Singh, R. S. and Ranganathan, B. (1974): Occurence of of Enteropathogenic ***E. coli*** serotypes in milk and milk products. Milchuissen-chaft. 29, (9): 529. Diry Sci. Abst. 37, 477.

Singh, R. S. and Ranganathan, B. (1977): Hygienic and public health significance of ***E. coli*** in milk and milk products. Indian Dairy Man, 29, 565. Dairy Sci. Abst. 40, 227.

Singh, S. P. and Singh, N. P. (1972): Studies on mortality in bovine calves due to pneumoenteritis. Ind. J. Anim. Sci., 42 (10), 798 – 802.

Sivaswamy, G. and Gyles, C. L. (1976): The prevalence of enteropathogenic Escherichia in the faeces of calves with diarrhoea . Can. J. Comp. Med., 40: 241 – 246.

Smith, H. W. and Lingood, M. A. (1972): Further observations on ***E. coli*** enterotoxins with particular regards to those produced by a typical piglets strains and by calf and lamb strains. J. Med. Microbiol., 5: 243 – 250.

Smith, K. E.; Stenzel, S. A.; Bender, J. B.; Wagstrom, E. and Taylor, C. M. (2004): Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day Comp. Pediator. Infect. Dis. J.; 23 (12): 1098 – 104.

Speicher, J. A. and Hepp, R. E. (1973): Factors associated with calf mortality in Michigan dairy herds. J. Am. Vet. Med. Assoc., 162 (6), 463 – 466.

Steiner, L.; Busato, A.; Burnen, S. A. and Gaillard, C. (1997): Frequency and atieology of calf losses and calves diseases before weaning in cow calf farms. II. Microbiological and parasitological diagnosis in diarrhoeic calves. Dtsch. Tieraztl. Wachenschr., 104 (5): 169 – 173.

Stephen, R. and Kuhn, K. (1999): Prevalence of verotoxin-producing Escheerichia coli mastitis and their antibiotic resistance patterns. 46 (6): 423 – 427.

Stephen, R.; Borel, N.; Zweifel, N.; Zweifel, C.; Blanco, M. and Blanco, J. E. (2004): First isolation and further characterization of enteropathogenic Escherichia col (EPEC) O157 : H45 strains from cattle. BMC. Microbiol. 17; 4: 10.

Stiles, M. E. and Lai-King, Na. (1981): Enterobacteriaceae associated with meat and meat handling – App. And Environmental Microbiology. 41, 4: 867 – 872.

Stone, G. G.; Oberst, R. D.; Hays, M.; McVeys, S. and Chengappa, M. M. (1994): Detection of ***Salmonella*** serovars from clinical samples by enrichment broth cultivation – PCR procedure. J. Clin. Microbiol., 32 (7): 1742 – 1749.

Svastova, A. (1980): Occurrence of some O-antigen of ***E. coli*** strains from calves with diarrhoea in the East Bohemian region. Vet. Med. 25, 329.

Talavera Coronel, A. (1981): O-group of ***E. coli*** strains isolated from newborn calves in cubs. CentroNacional de Salud-Animal, 1, 9.

Taylor, J. and Charter, R. (1952): The isolation of serological types of ***E. coli*** in two residen tial nurseries and their relation to infantile Gastroenteritis. J. path. Bacterial., 6: 715.

Thomson, B. M.; Cherry, W. B.; Davis, B. R. and Poales-Lebran, A. (1961): Rapid presumptive identification of enteropathogenicv ***E. coli*** in fecal smears by means of fluorescent antibody . Bull, Wld. Hitch. Org. 25: 137.

Tripathi, R. D. and Soni, J. L. (1983): Antibiotic sensitivity test with ***E. coli*** isolated from cases of neonatal calf diarrhoea . Indian Vet. J. 59, 413.

Tzpori, S. (1981): The atieology and diagnosis of calf diarrhoea . Vet. Rec. 108 (24): 514 – 515.

Tzipori, S.; Chandler, D. and Smith, M. (1983): The clinical manifestation and pathogenesis of enteritis associated with Rotavirus and enterotoxigenic ***E. coli*** infections in domestic animals. Prog. Food Nut. Sci., 7: 193 – 205.

Wary, C.; McLaren, I. And Carrall, P. J. (1993): ***Escherichia coli*** isolated from farm animals in England and Wales between 1986 and 1991. Vet. Rec., 13: 439 - 442.

Waltner-Toews, D.; Martin, S. W. and Meek, A. H. (1986): An epidemiological study of selected calf pathogen on Holestein dairy farms in South Western. Ontario. Can. J. Vet. Res., 50 (3): 307 – 313.

Wells, J. G.; Shipman, L. D.; Greene, K. D. et al. (1991): Isolation of ***E. coli*** serotype O157 : H7 and other Shiga-like toxin producing ***E. coli*** from dairy cattle. J. Clin. Microbiol. 29 (5): 985 – 989.

Wenneras, C. and Erling, V. (2004): Prevalence of enteropathogenic Escherichia coli-associated diarrhoea and carrier state in the developing world. J. Health. Popul. Nutr., 22 (4): 370 – 82.

Wierzba, T. F.; Abddel-Messih, T. A.; Abu-Elyazeed, R.; Putnarn, S. D.; Kamal, K. A.; Rozmjzl, P.; Ahmed, S. F.; Fatah, A. and Zabedy, K. (2006): Clinic-based surveillance for bacterial and rota virus-associated diarrhoea in Egyption children. Am. J. Trop. Med. Hyg.; 74 (1): 148 – 53.

Wolk, M.; Ohad.; Shpak, B.; Adler, H. and Nahari, N. (1992): A survey of enterotoxigenic ***E. coli*** from calves and lambs in the region of the Western Galilee in Israel during Winter 1980 – 1990. Israel Journal of Veterinary Medicine. 47 (1): 7.

Valente, C.; Fruganti, G.; Gardaras, P.; Morconi, F.; Kashari, Q. and Ciorba, A. (1982): Characterization of enterotoxigenic bovine ***E. coli*** isolated from newborn calves. Clinica Veterinaria, 105 (718); 257.

Vernacchio, L.; Verzina, R. M.; Mitchell, A. A.; Lesko, S. M.; Plant, A. G. and Acheson, D. W. (2006): Diarrhoea in American infants and youn children in the community setting: Incidence, clinical presentation and Microbiology. Pediatr. Infect. Dis. J.; 25 (1): 2 – 7.

Vidal, M.; Kruger, E.; Duran, C.; Lagos, R.; Levine, M.; Prado, V.; Toro, C. and Vidal, R. (2005): Single multiplex PCR assay to identify simultaneously the six categories of diarrhoea genic ***Escherichia coli*** associated with enteric infections. J. Clin. Microbiol.; 43 (10): 5362 – 5.

Viring, S.; Olsson, S. O.; Alenius, S.; Emanuelsson, U.; Jacobsson, S. O.; Larsson, B.; Linde, N. and Uggla, A. (1993): Studies of enteric pathogens and gamma-globulin levels of neonatal calves in Sweden. Acta Vet. Scand., 34 (3): 271 – 279.

Uysal, Y.; Erdogan, I.; Iavukcuoglu, F.; Gokcess, S.; Aslan, N. and Turutoglu, H. (1992): Detection of K99 , F41, F(4) and g 87 pillus antigens and heat stable enterotoxin (STa) and serotypes of ***E. coli*** strains isolated from newborn calves with colibacillosis . Pendik Veteriner Mikrobiyoloji Dergisi. 23 (2): 119.

Yalcin, N.; Gane, P.; Delahaye, J. and Mitton, A. (1969): Pathogenic role of ***E. coli*** and of Clostridia in calf mortality. Recl. Med. Vet., 145, 361 – 368.

Youssef, H. Y.; Hefnawy, S. H. Ahmed and Abd El-Rhaman, H. (1984): Bacteriological evaluation of raw minced meat in Assuit city. Fleischwirtsch, 64 (5): 590 – 592.

Zajicek, D.; Dvorackova, A. and Hawasi, K. (1986): Occurrence of Cryptosporidia in calves with diarrhoea. Sbornikvedeckych. Praci ustreduiho Statniho Veterinarniho ustavu, 16: 55 – 63.

Zeman, D. H.; Thomson, J. U. and Francis, D. H. (1989): Diagnosis, treatment and management of enteric ***Colibacillosis***. Vet. Med. 84 (8): 794 - 802.

Zhao, C.; Ge B.; De Villena, J.; Sudler, R.; Yeh, E.; Zhao, S. and Wagner, D. (2001): Prevalence of Compylobacter Spp., ***Escherichia coli***, and Salmonella serovars in retail chicken, turkey, pork, and beef from the Greater Washington. Appl. Environ. Microbiol; 67 (12): 5431 – 6.

ARABIC SUMMARY

الملخص العربى

جينات الضراوة فى الميكروب القولونى المعزول من الأغذية والحيوانات والإنسان

تعتبر الاسهالات وأمراض القولون من أكثر الامراض المسببة للخسائر الاقتصادية حيث تسبب زيادة فى عدد الوفيات ونقص فى الاوزان مع تأخير فى النمو فى العديد من الحيوانات حتى الانسان مع كثير من المخاطر فى الانسان والحيوان.

تعتبر الاشريشيا القولونية من أهم أنواع البكتيريا عالية الضراوة المسببة للاسهالات وأمراض القولون كما أن الايشريشيا القولونية بالاضافة لبعض الميكروبات الداخلية المعوية الاخرى من أكثر انواع البكتيريا المسببة للنفوق فى الحيوان وأشدها خطورة على صحة الانسان.

إستهدفت هذه الدراسة الى عزل وتصنيف الايشريشيا القولونية فى الانسان والحيوان وكذلك عينات الغذاء المتحصل عليها.

أيضا إستهدفت تلك الدراسة تشخيص وعزل الايشريشيا القولونية شديدة الضراوة والمفرزة لبعض السموم فى الانسان والحيوان وذلك عن طريق تفاعل البلمرة المتسلسل.

أجريت هذه الدراسة على عدد ٦٠ عينة متمثلة فى شكل مسحات قولونية مجمعة من العجول والاغنام من مناطق مختلفة بمحافظة كفر الشيخ ،وأيضا على عدد ٤٢ مسحة قولونية مجمعة من الاطفال وعدد ١١٢ عينة مجمعة من أغذية مختلفة على النحو التالى (٦٠ عينة ألبان ، ٣٣ عينة من الجبن ، ١٩ عينة من اللحوم).

تم تجميع العينات وترقيمها ووضعها فى أحواض ثلجية لحفظ العينات بدون أى تلف لها وتم إرسالها الى معمل الميكروبيولوجيا بكلية الطب البيطرى – جامعة الاسكندرية وذلك فى الفترة من مارس الى ديسمبر ٢٠٠٥.

وإستهدف الفحص البكتيريولوجى تصنيف ومعرفة خصائص البكتيريا المسببة للاسهالات وذلك من العينات المجمعة المختلفة من الانسان والحيوان والاغذية.

تم تشخيص وتصنيف البكتيريا المعزولة عن طريق الخصائص المورفولوجية، وصفات الاستزراع الخاصة بكل نوع من أنواع البكتيريا ، تشخيص الحركة للبكتيريا وأيضا من خلال الاختبارات البيوكيميائية.

أيضا تم عزل وتصنيف البكتيريا شديدة الضراوة عن طريق تفاعل البلمرة المتسلسل على البكتيريا المعزولة من العينات المجمعة.

وكانت أهم النتائج المتحصل عليها من تلك الدراسة هى:-

١-تم عزل الايشريشيا القولونية الشديدة الضراوة من عدد ١٠ عينات أى بنسبة ٣٣,٣ % و من عدد ٨ عينات أى بنسبة ٢٦,٧ % من عدد ٦٠ عينة (٣٠ عجل و ٣٠ حمل).

٢- كما تم عزل الايشريشيا القولونية شديدة الضراوة من ١٠ عينات أى بنسبة ٢٣,٨ % من المسحات القولونية المجمعة من أطفال تعانى من الاسهال أى وعددها ٤٢ عينة أى بنسبة ٢٣,٨ %.

٣- كما تم أيضا عزل الايشريشيا القولونية شديدة الضراوة من ١٠ عينات ، ٧ عينات ، ٩ عينات أى بنسبة ١٦,٧ % ، ٣٦,٨ % ، ٢٧,٣ % من عينات اللحوم ، و الالبان والجبن والتى عددها ١٩ ، ٦٠ ، ٣٣ على التوالى لنوعية الاغذية السابق ذكرها.

٤- من أستزراع عدد ٥٤ عترة معزولة من الايشريشيا القولونية على الوسط الدموى حيث وجد أن هناك ٥ عترات معزولة بنسبة ٩,٢٥% تقوم بعمل تحلل دموى من النوع ألفا وأن ٤٩ عترة معزول ليس لها أية تحللات دموية من النوع ألفا بنسبة ٩٠,٧٥% .

٥- أيضا أوضحت النتائج أن الـ ٥ عترات المعزولة والتى تقوم بعمل التحلل الدموى من النوع ألفا عزلت على النحو التالى عترتان من الاغنام بنسبة ٢٥% عترة واحدة من الانسان بنسبة ١٠% ، وعترتان من الالبان بنسبة ٢٠% على التوالى .

٦- أوضحت نتائج العزل والتعريف بخصائص البكتريا الناتجة من تفاعل البلمرة المتسلسل أن للايشريشيا القولونية التى تحمل الجين SLT-II هى ١٠ عترات من ١٠ عينات بنسبة ١٠٠% ، حيث تم تعريض ١٠عينات من العينات الموجودة (٣ عينات من العجول و ٢ عينة من الحملان و ٢ عينة من الاطفال وعينة واحدة من كل من اللحوم والالبان والجبن) إلى تفاعل البلمرة المتسلسل .

الخـــــــــــــلاصة

أوضحت هذه الدراسة أن الايشريشيا القولونية هى من أهم البكتيريا المسببة للاسهالات والامراض القولونية التى تصيب الحيوانات والانسان وتنتقل الى الانسان عن طريق الاغذية وخصوصا اللحوم والالبان .

كما أوضحت الدراسة أيضا أن تفاعل البلمرة المتسلسل من أهم الاختبارات والتفاعلات التى يمكن عن طريقها تشخيص وتصنيف الايشريشيا القولونية الشديدة الضراوة بالنسبة للانسان والحيوان .

جينات الضراوة فى الميكروب القولونى المعزول من الأغذية والحيوانات والانسان

مقدمة من

ط٠ب/ أنيسة عبد الله عوض سالم
بكالوريوس العلوم الطبية البيطرية
كلية الطب البيطرى جامعة الأسكندرية

للحصول على درجة
الماجيستير فى العلوم الطبية البيطرية
ميكروبيولوجيا (بكتريولوجيا)

لجنة المناقشة والحكم على الرسالة

موافقون

الأستاذ الدكتور / أحمد محمد عمـــــــــار
أستـــــاذ الميكروبيولوجيـــا المتفـرغ
كلية الطب البيطرى – جامعة الزقازيـق

الأستاذ الدكتور / حلمى أحمد تركـــــــــى
أستـــــذ الميكروبيولوجـــيا المتفـــرغ
كلية الطب البيطرى – جامعة الأسكندريـة

الأستاذ الدكتور / عباس أمين أحمـــــــــد
أستاذ الرقابة الصحية على الأغذية المتفرغ
كلية الطب البيطرى – جامعة الأسكندريـــة
(والمشرف على الرسالـــــــة)

الأستاذ الدكتور / سامى عبد السلام خليـــــل
أستاذ الميكروبيولوجيـــــــــــــا
كلية الطب البيطرى – جامعة الأسكندريـــة
(والمشرف على الرسالـــــــة)

التاريخ ٢٠٠٨/١/١٤

لجنـــــــــة الإشـــــراف

أ.د/ عباس أمين أحمد

أستاذ الرقابة الصحية على الاغذية

كلية الطب البيطرى – جامعة الإسكندرية

أ.د/ سامى عبد السلام خليل

أستاذ الميكروبيولوجيا

كلية الطب البيطرى – جامعة الإسكندرية

٤٤٣

جينات الضراوة فى الميكروب القولونى المعزول من الأغذية والحيوانات والإنسان

رسالة علمية

مقدمة إلى الدراسات العليا بكلية الطب البيطرى – جامعة الإسكندرية

إستيفاء للدراسات المقررة للحصول على درجة الماجستير فى العلوم الطبية البيطرية

تخصص

الميكروبيولوجيا

مقدمة من

ط.ب/ أنيسة عبد الله عوض سالم

(بكالوريوس العلوم الطبية البيطرية – جامعة الاسكندرية ٢٠٠٢)

(٢٠٠٨)