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The Health Implication of Enterohaemorrhagic *Escherichia coli* (EHEC) 0157:H7: A Review on Haemolytic Uraemic Syndrome

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Consumption of foods, water, vegetables, fruits, undercooked/ground/raw meat, unpasteurized milk or milk products contaminated with the bacterium strain *Escherichia coli* 0157:H7 has become a serious public health concern. This strain naturally inhabits the digestive tract of healthy cattle, and is released into the environment through the faeces of the animal. This strain cause haemorrhagic enterocolitis or gastroenteritis, and then haemolytic uraemic syndrome (HUS). HUS is a disorder characterised by haemolytic anaemia, low platelet count and acute kidney failure, and this disorder is a consequence of the production and action of Shiga-like toxin produced mainly by this bacterial strain (accounting for 90 percent of all cases), and occurs mainly in children less than five (5) years of age, but also occurs in the elderly. After infection with this bacterial strain, the disorder begins with intestinal perforation and ulceration leading to bloody diarrhoea, and consequently acute kidney injury, thrombocytopenia and microangiopathic haemolytic anaemia. In conjunction with clinical manifestations, several laboratory investigations (haematological, biochemical and microbiological assays) are implicated in the diagnosis of HUS. There is currently

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no specific treatment for HUS; however, supportive care (such as treatment of hypertension, fluid and electrolyte imbalance, haemodialysis, blood transfusion, etc) happens to be the only ameliorative measure for this disorder.

Keywords: Enterocolitis; haemorrhagic; diarrhea; disorder; haemodialysis.

1. INTRODUCTION

Haemolytic uraemic syndrome (HUS) is a disorder characterised by non-immune haemolytic anaemia, thrombocytopenia and acute kidney failure, occurring mainly in children less than five (5) years of age, but also in the elderly. It happens to be one of the commonest causes of acute renal dysfunction in children, with its clinical findings resulting from thrombotic microangiopathy (TMA).

Of all causes (such as some drugs, HIV infection, complement mutation, autoimmune diseases, malignancies, pregnancy, genetic factors, hypertension, etc), EHEC-induced HUS is the commonest type, accounting for approximately 90 percent of all cases [1]. Although, some strains of *Shigella dysenteriae* (such as *S. dysenteriae* type 1) and *Streptococcus pneumonia,* as well as some strains of *Escherichia coli* (such as 0104: H4, 026, 0145, 0103, etc) may induce the development of HUS [2], the 0157:H7 (EHEC) is the main strain implicated in the development of this disorder [3]. These strains of organisms induce HUS through the production, and thus action of a toxin referred to as Shiga toxin, also called Verotoxin (due to their cytotoxic effects on Vero cells) [4]; thus they are collectively referred to as Shiga toxin-producing *Escherichia coli* (STEC) [1].

The Shiga toxin-producing *E. coli* (STEC) or EHEC-induced HUS is also referred to as the outbreak HUS, and presents with abdominal pain, nausea, vomiting and bloody diarrhoea, which finally progresses into haemolytic anaemia, low platelet count and acute kidney injury [1]. The enterohaemorrhagic *E. coli* (EHEC) strain 0157:H7 being the main cause of HUS, was first implicated in enterocolitis during an outbreak in the United States of America in the year 1982 [5], and is naturally present in the digestive tract of healthy cattle; thus this animal serves as a vector for enterohaemorrhagic *E. coli*, which in turn is introduced into the environment through the animal's faeces. Another potential vector of this strain of *E. coli* is the common house fly *Musca domestica* [6]. Infection in humans however, occurs after consumption of contaminated undercooked meat or ground beef, vegetables, water, fruits, juice [7], unpasteurized milk [8] or milk products [9], from contaminated swimming pool, from animal to human, or from person to person via a faeco-oral route.

The incidence of haemolytic uremic syndrome depends on certain factors such as the temperature of the environment, locality, lifestyle of the host, etc. For example it occurs more in rural than urban areas, and more during summer than winter months [10]. Extensive outbreaks have been recorded in Germany and Japan [11], while smaller outbreaks have been recorded in some other countries [12]. Furthermore, the incidence in Argentina has been reported to be 12.2 cases per 100,000 children below 5 years of age [13], and this may be attributed to their higher rate of consuming raw meat. In general however, an incidence of 6 per 100, 000 children below 5 years of age have been reported [14].

2. EPIDEMIOLOGY

HUS due to enterohaemorrhagic *E. coli* (EHEC) 0157:H7 (or Shiga toxin-producing E. coli) occurs primarily in children younger than 5 years of age, and also in the elderly [15]. The yearly incidence of HUS in the North America and Western Europe is about 2-3 per 100,000 younger than 5 years of age [2]. This EHEC-induced HUS is responsible for 90 percent of cases in childhood [16], and occurs more in summer months. After an incubation period of 2 to 5 days, patients infected with EHEC develop gastroenteritis/enterocolitis with consequent diarrhoea which may be watery, but later becomes grossly bloody [6]. About 10 to 15 percent of children with EHEC colitis develop HUS within 13 to 14 days [17,16].

The natural reservoir of enterohaemorrhagic *E. coli* is the digestive tract of healthy cattle, and infection may be food and/or water-borne and environmental [18]. The mode of infection is through the consumption of contaminated foods, fruits, undercooked contaminated vegetables and meat (particularly ground beef), unpasteurized milk, juice, cross-contamination of food products

and cooking utensils, contaminated swimming pools, person to person or animal to person [19], and is thus a major concern in day-care centres. Infection with this strain of *E.coli* requires a very low number of bacterial organisms [20], with infectious dose appearing to be only 500 bacteria [6].

3. *E. coli* 0157:H7 SHIGA TOXINS

Shiga toxins also called Verotoxins (so called due to their cytotoxic effect on Vero cells) [4] are a family of exotoxins derived from the bacterial strain *E. coli* O157:H7; although it may also be produced by other bacterial types. These toxins play a vital role in the pathogenesis of haemolytic uremic syndrome by directly inducing microvascular endothelial cell damage in specific target tissues, such as the kidney, due to the expression of the toxin-specific receptors on the surfaces of endothelial cells [21].

There are two main types of Shiga toxins, Shiga toxin 1 (Stx 1) and Shiga toxin 2 (Stx 2), with the Stx 2 being more frequently occurring in bacteria, causing HUS than Stx 1 [22]. They are A:B5 toxins, consisting of a pentameric receptor binding B subunit and a single enzymatically active A subunit, and act to inhibit protein synthesis through binding to its glycosphingolipid receptor, globotriaosylceramide (Gb3) on the target cell surface [23].

After binding to its cell-surface receptor, Shiga or Vero toxin undergoes retrograde transport within the cell. The active A subunit is transported from the endoplasmic reticulum to the ribosome, where it cleaves an important adenine base, A4565, from the human 28S rRNA, thus preventing peptide elongation in the host cells [24].

4. PATHOPHYSIOLOGY

Following infection, the bacteria resist the acidic pH of the stomach, and enter into the intestine where they interact with other intestinal microbes (microflora) in a process called quorum sensing to create intestinal colonization [25] initially in the terminal ileum [26], and then in the colon. The bacteria then attach their fimbriae to the surface of colonic enterocytes, express its translocated intimin receptor (Tir), and translocate this Tir to the enterocyte membrane. The bacteria also express their intimin (Bacterial Intimin) which binds to the Tir located on the enterocyte; this binding enables the bacteria to be more firmly attached, generating an ‘‘attaching and effacing lesion’’ [27], and thus release Shiga toxins in the intestine.

The Shiga toxins contain a pentameric B subunit and a single enzymatically active A subunit; on the surface of the enterocytes (on intestinal epithelial cells or Paneth cells), the pentameric B subunit binds to its glycosphingolipid receptor, Globotriaosylceramide (Gb3), enabling entry of the holotoxin into the enterocytes through a process of endocytosis and possibly by macropinocytosis [28]. Within the enterocytes, the Shiga toxin undergoes retrograde transport, with the enzymatically active A subunit being translocated from the endoplasmic reticulum to the ribosome, where it binds to the ribosomal RNA and inhibits protein synthesis [29], resulting in intestinal cell apoptosis, and haemorrhagic colitis. This may further promote release of Shiga toxins and bacterial colonization by means of quorum sensing [30].

From the intestinal cells, the Shiga toxins cross the mucosal barrier and enter into the blood circulation [31] where most of it binds to Gb3 receptors on the surface of blood cells (such as neutrophils, monocytes, platelets and red blood cells) [32], and gets circulated in the bloodstream, followed by entry of the Shiga toxins into the blood cells [33], resulting in the activation of platelets and leucocytes by the toxin [34]. Blood cells (such as platelets and red blood cells) that lack protein synthesis are not negatively affected by the toxin [33].

These blood cells form microvesicles (now containing the Shiga toxin), followed by the release of the microvesicles into the bloodstream, which are then carried to the kidneys, and may be taken up by glomerular and peritubular capillary endothelial cells [1]. Within the renal cells, the toxin is released, and gets bound to its Gb3 receptor on the surface of the glomerular and peritubular capillary endothelial cells. The toxin then enters into these cells by endocytosis, followed by the transportation of the enzymatically active A subunit in a retrograde manner to ribosomal RNA [35], leading to the inhibition of protein synthesis and thus injury to, and inflammation and death of the endothelial cells; this endothelial cell injury inhibits prostaglandins and prostacyclins, and thus activates thromboxanes which induce platelet aggregation, resulting in the formation of microthombi in the injured areas within the endothelial capillaries. Therefore, the blood

vessels become partly occluded with the microthrombi, such that circulating erythrocytes are forced through these occluded vessels, leading to the deformation and fragmentation of the erythrocytes, thus producing schistocytes, which are removed from the circulation by the reticuloendothelial system (RES), resulting in haemolytic anaemia; thus the term microangiopathic haemolytic anaemia (MAHA). Thrombocytopenia may be due to the large numbers of platelets used up for the formation of the microthrombi [35].

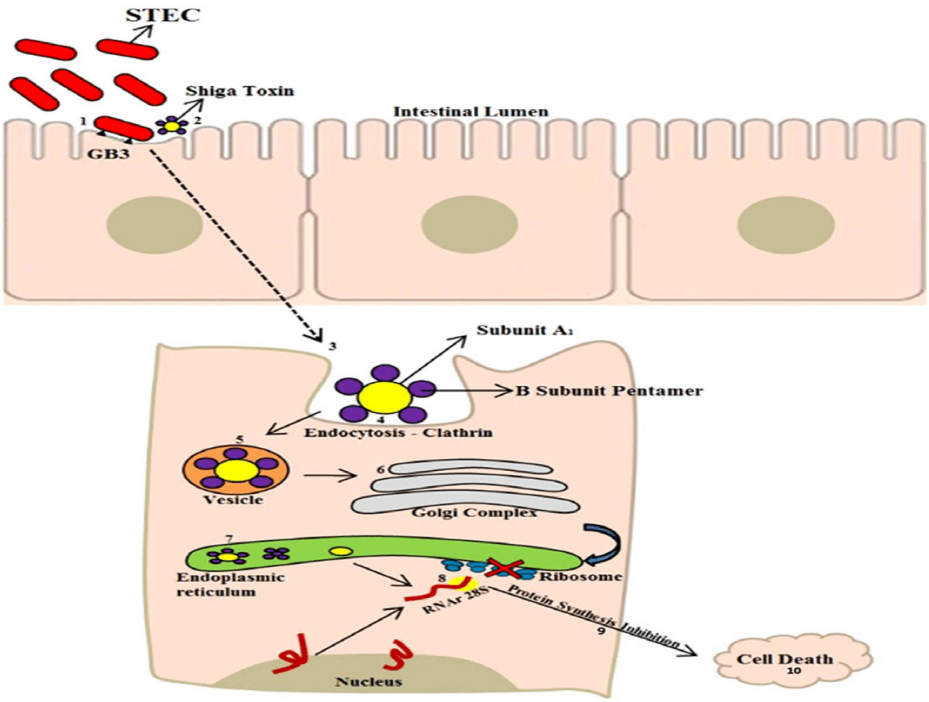

Fig. 1. Pathophysiology of enterocolitis induced by shiga toxin
Source: www.google.com

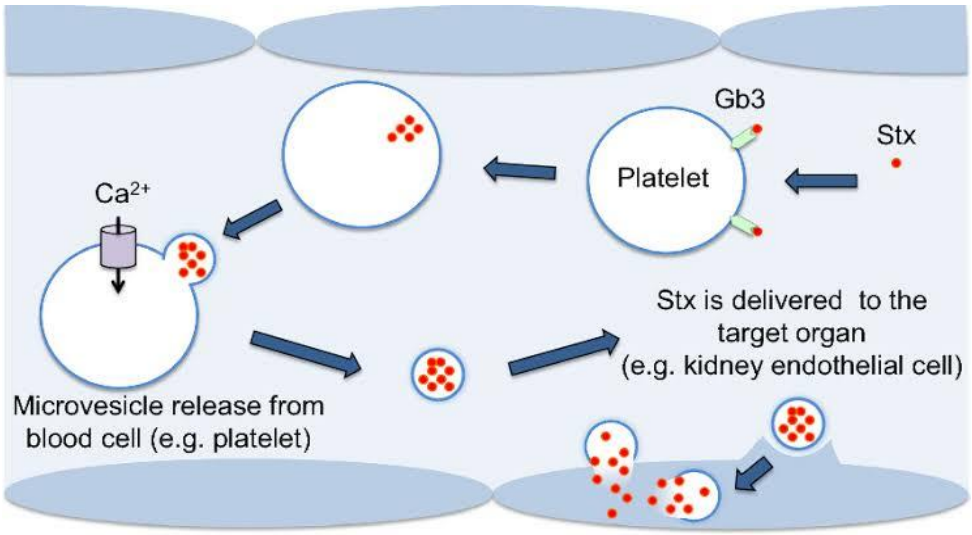

Fig. 2. Mechanisms of action of shiga toxins in the systemic circulation
Source: [8]

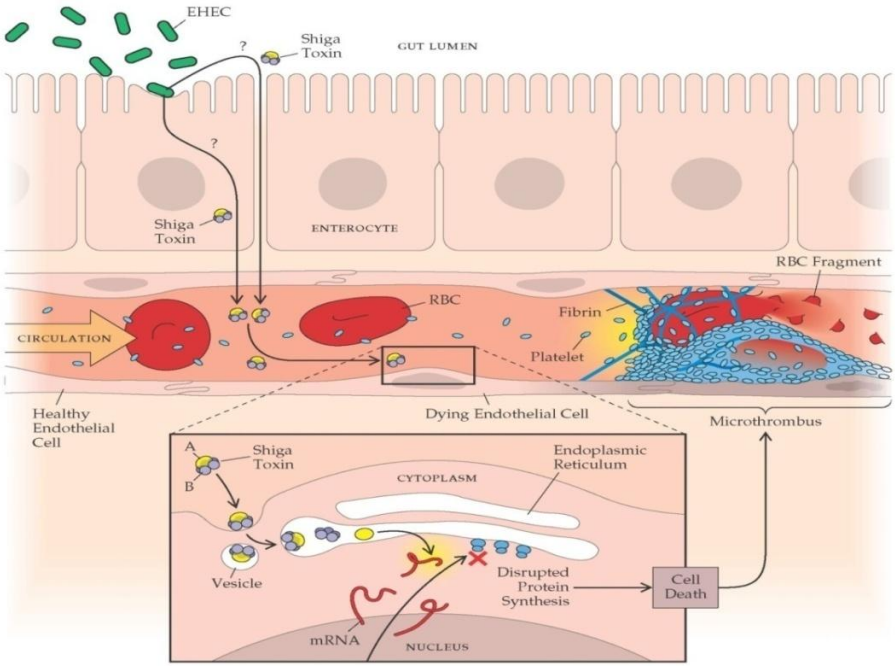

Fig. 3. Pathophysiology of HUS; showing the process of enterocolitis, and formation of microthrombus and RBC fragments

Source: www.google.com

5. CLINICAL MANIFESTATIONS

The clinical manifestations begin with an episode of severe abdominal cramps, nausea, vomiting and bloody diarrhoea; indicating the digestive tract involvement, with manifestations such as severe haemorrhagic colitis, bowel necrosis and perforation, rectal prolapse, peritonitis, and intussusceptions [36]. Fever may be absent, but if present, it is mild. The triad clinical manifestations of HUS include the following:

5.1 Microangiopathic Haemolytic Anaemia (MAHA)

Red blood cells passing through the renal capillaries that have been partially obstructed or occluded with microthrombi, are mechanically destroyed, resulting in the deformation of the normal shape of the red blood cells, which further results in the fragmentation of the cells, leading to the formation of schistocytes, which are in turn are removed from the circulation by the reticuloendothelial system. Alternatively, [37] stated that erythrocytes may become fragmented due to oxidative damage resulting from alterations in the metabolism of glutathione as seen in a study with HUS patient. Thus, patients with Shiga toxin-induced HUS exhibit reticulocytosis during the acute phase of the disease [8]. Therefore, with 3 to 14 days after the onset of the bloody diarrhoea, the patient presents with signs of anaemia such as pallor with or without jaundice [35].

5.2 Thrombocytopenia

Thrombocytopenia (low platelet count) in HUS occurs due to platelet activation and deposition of aggregates (microthrombi) along the damaged vascular wall. Platelet is activated when the subendothelium (after toxin-induced endothelial cell damage) is exposed allowing platelets to interact with fibrinogen, collagen and von Willebrand factor to form aggregates [38].

Also, platelets are activated directly by Shiga toxin and 0157:H7 lipopolysaccharide (LPS) [39] and by cytokines released by activated monocytes or endothelial cells [40]. They play a role in the inflammatory process through interaction and forming complexes with leucocytes and through the release of pro-inflammatory cytokines [41], and also form microangiopathic lesions during haemolytic uraemic syndrome; low platelet counts are correlated with the degree of renal dysfunction [42]. Despite the thrombocytopenia however, purpura or active bleeding does not occur [43].

5.3 Acute Kidney Disease (AKD)

The Shiga toxin enters the kidneys and affects the glomerular (endothelial cells, podocytes and mesangium) and tubular cells [44], causing acute renal injury. Therefore, acute renal failure is attributed to the Shiga toxin-induced vascular injury in the endothelial capillaries of the glomeruli, triggering the formation of occluding microthrombi, as well as acute toxin-induced tubular injury [45].

The renal involvement may vary from mild to severe forms; in the mild form, patients only present with microscopic haematuria, minimal proteinuria and normal urine output, whereas in severe form, patients may present with oliguria or anuria, widespread renal cortical necrosis and irreversible renal failure [35]. However, all patients with HUS experience haematuria and proteinuria, except if they are anuric.

5.4 Other Manifestations

Other extra-renal manifestations may occur in HUS, although these manifestations are non-specific. They may include the following:

1. A variety of fluid and electrolyte imbalances due to reduced kidney function, haemolysis and tissue catabolism; including hyponatremia which could be due to the diarrhoea, hyperkalaemia which could be due to reduced glomerular filtration rate, haemolysis and tissue catabolism, and metabolic acidosis which could be due to reduced kidney function and tissue catabolism. Also present may be hyperphosphataemia and hypocalcaemia [35].
2. Fluid overloads (caused by reduced renal function) may cause oedema, hypertension, and thus cardiac failure [35].
3. The pancreas may be affected, leading to pancreatic inefficiency, and thus the development of transient diabetes mellitus [36].
4. The CNS may be affected, resulting in symptoms such as seizures (the most common), comma, brain oedema, cortical blindness, stroke, lethargy, irritability and hemiparesia [46].
5. The liver may also be affected resulting in hepatomegaly [47].

6. LABORATORY DIAGNOSIS

The laboratory diagnosis of haemolytic uremic syndrome (HUS) involves haematological, biochemical and microbiological assays, as well as urinalysis.

6.1 Tests to Diagnose Non-Immune Haemolytic Anaemia

These include:

- Full Blood Count (FBC), mainly to determine platelet count (to diagnose thrombocytopenia) and haemoglobin concentration, in which the platelet count is usually below 40, 000/mm^3, and the haemoglobin concentration below 8 g/dL [10].
- Direct anti-humanglobulin (Coombs) test, which is usually negative, indicating that the haemolysis is non-immune
- Serum lactate dehydrogenase (LDH) level, which is usually elevated
- Unconjugated bilirubin level, which is usually elevated.
- Haptoglobin level, which is usually decreased
- Peripheral blood smear, which shows schistocytes or helment cells (which are fragmented erythrocytes) and increased reticulocyte counts [8].

6.2 Biochemical Assays

These include:

- Serum creatinine, urea and electrolyte analysis, in which both the serum creatinine and urea levels are elevated, accompanied by hyponatraemia, hyperkalaemia, and metabolic acidosis (as indicated by low bicarbonate ion concentration or by elevated anion gap).
- In some cases, liver enzymes may also be analyzed to assess the liver (In most cases, liver enzymes are elevated) [8].

6.3 Microbiological Assays

These include:

1. Stool culture in sorbitol MacConkey Agar (SMAC), with colonies being colourless (does not ferment sorbitol, thus said to be sorbitol-negative) and non-haemolytic. The sorbitol-negative colonies selected from

SMAC should be sub-cultured to another medium (for example, blood agar) for 24 hours, and then the colonies from this medium should be tested with *E. coli* 0157:H7 antiserum (agglutination assay). If the agglutination assay is positive, it is a confirmation that the isolated organism is 0157:H7. However, biochemical tests should be performed on the colonies to be identified as *E. coli* (because other species can cross-react with 0157 antiserum) [48].

2. Besides the culture, serology testing may be done, in which an ELISA is used for EHEC virulence factors such as serotype-specific lipopolysaccharide, Shiga toxin or adhesins, and confirmation of *E. coli* 0157:H7 can be done by identifying the H7 flagellar antigen.
3. Testing for the *E. coli* 0157 strains for the enzyme B-glucuronidase using broth or agar medium containing the substrate 4-methylumbelliferyl-B-D-glucuronide (MUG) is also necessary. Usually, MUG is cleaved by this enzyme, resulting in a detectable fluorescent product. However, this strain lacks the enzyme, and is therefore MUG-negative [48].

6.4 Urinalysis

This involves the use of dipstick. Also, urine microscopy is done, giving rise to microscopic haematuria, proteinuria and casts revealing glomerular injury [1].

7. TREATMENT

There is no specific treatment for typical HUS. However, treatment involves supportive care which includes:

- Treatment of fluid and electrolyte imbalance: At the gastrointestinal phase of the disease, vomiting, diarrhoea and decreased fluid intake may cause dehydration. In this situation, fluid support with appropriate electrolyte may be given [10] orally, intravenously, or by tube [35]. When replacement fluids cannot correct fluid and electrolyte imbalances, or when fluid overload affects cardiac or pulmonary function, peritoneal dialysis or haemodialysis should be done. During this phase, intake of anti-motility drugs and antibiotics should be avoided, as this may increase the risk of developing HUS.
- Treatment of hypertension: Increased fluid loading should be corrected using diuretics such as furosemide. Hypertension should be treated to prevent hypertensive encephalopathy and congestive heart failure; this is done using short-acting calcium channel blockers such as nifedipine should be given orally, or nicardipine or nitroprusside should be given intravenously (In cases of hypertensive encephalopathy, intravenously treatment is preferred).
- Treatment of symptomatic uraemia (for example uraemic encephalopathy), azotaemia (BUN greater than or equal to 100 mg/dL), and severe fluid loading (unresponsive to diuretics), involves dialysis.
- Treatment of anaemia is done through erythrocyte transfusion, particularly when the haemoglobin concentration is less than 7g/dL.
- Treatment of thrombocytopenia requires platelet transfusion only for patients with life-threatening bleeding, or during preparation for surgery [8].

8. ECONOMIC IMPACT DUE TO E.COLI 0157:H7 INFECTIONS

Infections due to *E. coli* O157 impose significantly great economic costs by incurring medical bills and negatively affecting the productive system of a society. The Centers for Disease Control and Prevention (CDC) gave an annual estimate of approximately 73,000 illnesses due to Shiga toxin–producing Escherichia coli O157 (O157 STEC) infections in the United States, leading to more than 2,000 hospitalizations and 60 deaths [49]. The yearly cost of sicknesses due to *E. coli* O157 was estimated to be 405 million dollars, which includes 370 million dollars for premature deaths, 30 million dollars for medical care, and 5 million dollars in lost productivity [49].

9. CONCLUSION

Although, haemolytic uremic syndrome is accompanied by several deleterious health effects, seventy percent of cases resolves, while thirty percent leads to permanent kidney damage and neurological pathologies. However, prevention of this disorder is possible through proper cooking of meat and vegetables before eating, proper treatment of water before drinking,

consumption of only pasteurized milk, and ensuring good personal hygiene.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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