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Escherichia coli O157:H7 Infection and Hemolytic Uremic syndrome among Iraqi Diarrheal Children

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Objectives: The first cases of *E.coli* O157: H7 infection in Iraq are reported in order to determine their association with Hemolytic Uremic syndrome (HUS).

Design:

Methods: Blood, urine, and stool samples were collected from 687 hospitalized children with diarrhoea in Basrah, Iraq. The causative agents (Bacteria and parasites) for diarrhoea were identified, especially *E.coli* O157: H7 which was subjected to serotyping by fluorescent technique. Direct smear; Ritchie formalin–ether–sedimentation concentration and modified Ziehl-Neelsen staining techniques were used to identify the parasitic infections in stool samples. General urine analysis and complete blood picture were studied as well.

Results: Eighteen (2.6%) children were found to-be infected with *E.coli* O157: H7, half of them (50%) developed HUS. The infection rate was higher among infant boys residing in rural areas. The duration of illness ranged from 2-14 days, with mild clinical presentation and was distinguished from other bloody diarrheal infections by the lack of fever. Receiving antimicrobial therapy has no meaningful effect in reducing the complication of HUS.

Conclusion: The present study provided useful information on the seasonal occurrence, age at risk, pattern of feeding among children<2 year of age and highlighting the importance of one of emerging infectious agents in Iraq.

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Escherichia coli O157: H7 is an emerging pathogen and a newly recognized as a cause of hemorrhagic colitis[1] and is associated with Hemolytic Uremic Syndrome (HUS)[2], a triad of acute renal failure, micro-angiopathic hemolytic anemia, and thrombocytopenia[3]. *Shigella, Salmonella typhi, Campylobacter jejuni*[4-6], and coxsackie virus[7] infections have been incriminated as causative agents of this syndrome.

This serotype of *E.coli* produce potent cytotoxins (verotoxins I & II)[2]. Elaboration of these toxins depends on the presence of certain phages carried by the bacteria, which have a plasmid that allows expression of a novel type of fimbria that is involved in attachment of

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the bacteria to intestinal mucosa[3]. Fibrin deposits, hemorrhage in the submucosa, mucosal ulceration, neutrophil infiltration, and microvascular thrombi are the pathological processes mediated by the *E.coli* O157:H7- shiga – like toxin[2,3].

E.coli O157:H7 first came to prominence in 1982 with two geographically separated outbreaks in the United States[1], since then outbreaks have been documented from a number of countries[8-9]. The largest outbreak ever reported occurred in the western state of Washington in 1993[9.] Seven hundred and thirty-two cases were identified, mostly children of whom 195 were hospitalized, 55 developed HUS and four died.

This is the first reported study for this emerging infectious agent causing diarrhoea in Iraq.

METHODS

Blood, urine and stool samples were collected from 687 children (320 males and 367 females), during the period from December 2001 to November 2002 from Basrah General Hospital, Basrah Teaching Hospital, and Pediatrics Hospital in Basrah. Their ages ranged from 3 months to 12 years with a mean of 5.41 ± 3.85 years.

All patients suffered from acute diarrhea, which was defined as a change in normal stool pattern for a period of not more than two weeks, characterized by an increase in the stool frequency of three or more times within the preceding 48 hours, and with an increase in the liquidity of stools. The study was conducted at Basrah Governerate (south of Iraq) and only hospitalized patients were eligible for inclusion. Children with a history of antibiotic use or gastroenteritis within the preceding two weeks were excluded. Other exclusion criteria were a history of other significant gastrointestinal diseases such as inflammatory bowel disease, known immunodeficiency states, and other chronic diarrheal illnesses.

A structured questionnaire was used for data collection and information was obtained both from the children hospital case-notes and from an interview with the child's mother or his care giver. Wherever possible, sample of the first stool passed was sent directly to the laboratory, and in all cases samples were collected within 48 hours of admission, to reduce the chance of hospital – acquired infections.

Stool samples were innoculated for *Salmonella, Shigella, Klebsiella Oxytoca, Yersinia enterocolitica, Campylobacter* (on suitable media for each), and on sorbitol MacConkey agar for *E.coli* O157:H7 (Which is negative for sorbitol fermentation)[(10)]. All presumptive *E.coli* O157:H7 isolates were subjected for serotyping by using the Oxiod O157 direct fluorescent antibody conjugate of Kirkegaard and Perry (Kirkegaard and Perry Laboratories Inc., Gaitherburg , Md) for O157 Antigen, and H7 antiserum (Difco Laboratories, Detroit, Michigan) for H7 antigen.

All stool samples were also subjected for direct smear method and then Ritchie formalin-ether sedimentation concentration technique[11] for detection of ova, cyst and trophozoite stages of parasitic infections including *Giardia lamblia, Blastocystis hominis, Entamoeba histolytica, Entrobias vermicularis, Trichuris trichiure, Cryptosporidium sp., Cyclospora sp.,* and *lsospora belli*. Few drops of the sediment were subjected to modify Ziehl-Neelsen staining technique to detect acid-fast protozoal parasites including the last three organisms[11].

Blood samples were collected for Hb, platelet count, leucocyte count, plasma haemoglobin, serum haptoglobin, bilirubin, serum creatinine, and urea nitrogen. Urine samples were examined microscopically after centrifugation for casts, epithelial, RBC, and leucocyte cells.

RESULTS

Out of 678 samples *E.coli* O157:H7 were found to be excreted in 18 (2.65%) children, (Table 1). Infection rate was higher (3.84 %) among children <2 years of age than other age groups which began to decrease to1.81% in 10-12 years age group. That may represent the decline curve of the infection in relation to age.

Table 1. Number and percentage of Isolated diarrheal causative agents among 678 patients and HUS positives

		Isolated diarrheal causative agents							
		*E.coli*0157:H7		Others		Total		HUS	
Age groups (year)	No Examined	No	(%)	No	(%)	No	(%)	No	(%)
<2	182	7	3.84	32	17.58	39	21.42	3	1.64
2-4	160	5	3.12	35	21.87	40	25.00	3	1.87
5-9	171	3	1.75	25	14.16	28	16.37	2	1.16
10-12	165	3	1.81	14	8.43	17	10.30	1	0.60
Total	678	18	2.65	106	15.63	124	18.28	9	1.32

X= 5.41 SD=3.85

Causative agents other than *E.coli* O157: H7, which were recovered from 106 stool samples (15.63%), seemed to draw the same curve as in the *E.coli* O157: H7 infection in relation to age.

All the 18 culture-confirmed patients resided in Basrah Governorate with no history of travel to any endemic area. The disease occurred in 2 cases in the winter of 2001 (December through February), 4 cases in the spring of 2002 (March through May), and 12 cases in the summer of 2002 (June through October).

Children testing positive for *E.coli* O157:H7 are shown in Table 2. Their age ranged from 5 months to 11.8 years with a mean of 4.24 ± 3.92 years. The boy-to-girl ratio was 1.25:1, similar to the ratio for rural-to-urban types of residency. The incidence rates of the infection among seven children below 2 years in relation to three types of feeding were; 14.2% (1/7) in breast feeding; 57.1% (4/7) in bottle or artificial milk; and 28.5% (2/7) for mixed types of feeding.

The mean duration of illness for the 18 patients was 8.2 days (range 2 to 14 days). All patients were hospitalized and the mean duration of hospital stay was 7.5 days (range 2 to 17 days). Most patients complained of vomiting, abdominal cramping, and were clinically dehydrated. Three developed HUS, 7 days after onset of non-bloody diarrhea and in four patients HUS developed four days after the onset of bloody diarrhea, caused by *E.coli*

O157: H7. The other two patients met the case definition and the stool specimen yielded *Campylobacter* and *S.taphi*, they developed the syndrome five days after the onset of watery and mucoid diarrhea.

All patients received IV rehydration with a mean of 1.8 liters/person/day. Sixteen of the 18 patients received a mean of two different antimicrobial agents, the median duration of antimicrobial therapy was five days. Eight patients, received anti-diarrheal agents after 1-2 days of starting the symptoms. Six patients received blood transfusion and recovered without sequelae, none required dialysis. All *E.coli* O157: H7 isolates were sensitive to chloramphenicol, trimethoprim-sulfamethoxazole, nalidixic acid, and two isolates were resistant to ampicillin.

Table 2. Details of the children testing positive for *E.coli*

Case No	Age Y/M	Sex	Res.	Type of feeding	Clinical features							HUS
					Dia	Vom	Dehyd	Fever	Cramp	Olig.	Pall	
1	3.2	M	R	Breast	Bloody	+	Severe	+	+	+	+	+
2	12	F	R	Breast	Bloody	+	Severe	+	+	-	+	-
3	4-	M	U	……	Mucoid	+	Mod	-	+	-	-	-
4	25	F	R	Both	Bloody	+	Mild	-	+	+	+	+
5	64	M	U	……	Mucoid	+	Mild	+	+	-	-	+
6	35	F	R	……	Mucoid	-	Mild	-	+	+	+	+
7	110	M	U	Bottle	Bloody	+	Mild	+	+	+	+	+
8	85	F	R	……..	Watery	-	Mild	-	-	-	+	-
9	-10	F	R	Both	Mucoid	+	Mod.	-	+	-	+	-
10	-5	M	U	Bottle	Bloody	+	Severe	_	+	+	+	+
11	111	F	U	Both	Watery	+	Mod.	-	+	+	+	-
12	113	M	U	……	Watery	-	Mod.	-	+	+	-	-
13	-9	M	R	Bottle	Bloody	+	Severe	-	+	-	+	-
14	-11	M	U	Bottle	Bloody	+	Mod.	-	+	+	-	-
15	105	F	R	…….	Bloody	+	Mod.	+	+	+	+	-
16	38	F	R	……	Watery	+	Mild	-	+	+	+	+
17	118	M	U	…….	Bloody	+	Mild	+	+	+	+	+
18	55	M	R	……..	Mucoid	-	Mild	+	+	+	+	+

Y: Year, M: Month, Res: Residency, R: Rural, U: Urban, Olig.:Oligurea, Pall.:Pallor.

Six boys and three girls (1.32%) developed HUS out of the 678 children with diarrhoea. Their laboratory values were: platelet count 10,000 to 80,000/ cumm. (mean 43,000/cumm.); Hb 6.6-11 g/dl (mean 8±1.7g/dl); leucocyte count 7600-17,400/mm^3 (mean 1900 mm^3), neutrophils displayed slight to moderate shifting to left; plasma HUS from 5-8mg/dl (mean 6.38±28 mg/dl); Bilirubin from 2.5-6 mg/dl (mean 3.8±1.07 mg/dl); serum creatinine 1-4.5 mg/dl (mean 2.97±1.08 mg/dl); Bloodurea nitrogen 35-101mg/dl

(mean 24.7±61.88mg/dl); casts in urine 2-5 plus (mean 4+). All had a micro-angiopathic hemolytic anemia with schistocytes present in blood smears.

DISCUSSION

Infections caused by *E.coli* O157: H7 and other verotoxigenic *E.coli* have emerged as a major public health concern in North America and Europe[3,8,9]. Our positive results were significantly lower than expected values, which may be attributed to the single stool samples collected from patients, hence, there may be underestimation of the incidence rate because of unknown shedding nature of the organism.

Raw and pasteurized milk-borne transmission was considered as a potent source of infection[10,12] and regular isolation of the organism from feces of healthy cattle[13], may explain the highest rate of infection among rural residents than those from urban areas.

Males in the present study were more prone to the infection, that may related to the fact; males are more active, mobile, and integrated in the environment specially among agricultural community. That is in agreement with findings reported elsewhere[2,3], but it is in contrast with others[5].

Protective effects of breast feeding (non exposure to contaminated milk or protective properties including antibodies) was suggested in this study, hence the absence of breast feeding and using bottle only (artificial milk) might increase the probability of the infection among children <2 years age.

Although the principal source of infection could not be identified in this study, the highest rate of infection was observed in the summer of 2002. This could be attributed to the general unavailability of good drinking water in their community in the hot season and the inhabitants of Basrah must obtain their drinking water by storing it in containers which increases the exposure to contamination by microorganism.

The clinical illness caused by *E.coli* O157: H7 observed in the present study was milder than that noticed previously[2,3]. Bloody diarrhea is not an invariable finding, 44% were identified as having non bloody diarrhea.

Clinical presentation of this illness may be distinguished from others of bloody diarrhea which were described in shigellosis, amoebiasis, campylobacteriasis, or invasive *E.coli* gastrointeritis by the lack of fever and the bloody discharge resembling lower gastrointestinal bleeding[14,15].

This finding suggests that people with diarrhea seek medical care infrequently; they do only when the illness is severe or prolonged, and are unlikely to be tested for *E.coli* O157: H7 infection. In the present study *E.coli* O157: H7 ranked fifth as a diarrheal causative agent after *Blastocystis hominis, Giardia lamblia, Entamoeba histolytica, Cryptosporidium sp.*

Unlike the detection of other intestinal pathogens which are identified by standard routine stool examination, the diagnosis of *E.coli* O157: H7 requires the detection of toxins as well as the bacteria itself[10]. The presence of shiga-like toxin can be detected by demonstrating cytotoxicity, dot-blotting, Polymerase Chain Reaction or by ELISA[10-16].

The course and prognosis of HUS differs substantially between adult and children[8]. A number of etiological factors associated with its development suggest that HUS in childhood commonly follows an infection process, usually with a prodrome of vomiting, oliguria, and diarrhea[17]. The acute renal failure of HUS is related to small-vessel occlusion and glomerular subendothelial deposits. If the latter predominate, recovery is faster and more complete than when small arterioles are involved[18]. Thrombocytopenia in our patients was probably due to adhesion of platelet to glomeruler basement membrane[4,18]. Six patients needed blood transfusion because of severe anemia which was presumably due to mechanical fragmentation of red blood cells by vessels diseases by schistocytes and burr cells[17-19].

Other laboratory findings revealed that leucocyte count was commonly raised and there may be a leukaemoid reaction[10,17]. Serum biochemistry revealed evidence of hemolysis and acute renal failure with markedly raised blood urea and serum creatinine, proteinurea, hematuria and urinary casts were also present. None of the patients needed dialysis.

The majority of patients in our study received antimicrobial therapy according to antibiotic sensitivity test whereas in previous studies most patients did not[1,14]. In their 17 years survey of HUS[3], the authors found no significant change in the complication rate despite treatment by antibiotics. However, the duration of illness in the present and previous studies was similar, that may suggest the antimicrobial therapy is ineffective. Recently, therapeutic plasma exchange was used[20] to reduce the mortality rate of the infection in the Lanarkshire outbreak in 1996, but this controversial treatment was used for adults and was expensive. Further studies are needed to investigate the HUS complications and their prevention especially to the two extremes of age (<4 years and >65 years) which are considered as risk factors. The use of anti-motility agents may lead to increase in the infection by allowing the multiplication of the organism and increased production of verocytotoxin.

CONCLUSION

Hemolytic Uremic Syndrome tends to occur either sporadically or in micro-epidemics chiefly among infants younger than 2 years of age. The association of this syndrome with *E.coli* O157:H7 infection is of interest to epidemiologists because of the many outbreaks caused by it. Thus, further studies are needed to reduce the mortality and morbidity rate.

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