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Enterotoxigenic *Escherichia coli* Food-Borne Disease Outbreaks in Yazd Province of Iran during 2012-2016

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HIGHLIGHTS

- Enterotoxigenic *Escherichia coli* (ETEC) outbreaks in Ashkezar, Mehriz, and Taft were more prevalent than the other cities of Yazd province.
- There was a positive relationship between suspended dust condition and the incidence rate of ETEC outbreaks.
- Some associations were found between incidence rate of ETEC outbreaks and demographic features.

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Acronyms and abbreviations
ETEC=Enterotoxigenic *Escherichia coli*
IR=Incidence Rate

ABSTRACT

Background: Enterotoxigenic *Escherichia coli* (ETEC) is one of the most important agents of travelers' diarrheal diseases in the developing countries. The main purpose of this study was to determine the association of ETEC outbreaks with climatic and demographic variables in Yazd province of Iran.

Methods: This study was done on 729 food-borne disease rectal swab samples, which gathered during 48 ETEC outbreaks in Yazd province from 2012 to 2016. The isolates were identified by biochemical tests, serotyping, and heat labile enterotoxin assays in Vero cell line culture. The climatic data was gathered from Iran's Meteorological Organization and Yazd synoptic stations. Data were analyzed by Stata statistical software.

Results: The rates of ETEC outbreaks in Ashkezar, Mehriz, and Taft were significantly ($p<0.05$) more than the other cities of Yazd province. A positive relationship was found between suspended dust condition and the IR of ETEC outbreaks. The IR of ETEC outbreak in autumn was more than the other seasons.

Conclusion: The present work showed the association of ETEC outbreaks with some factors such as demographic features, location status, and climate variations.

Introduction

Food-borne disease outbreak events occur if more than two people eat the same food and appear the same illnesses with common apparent symptoms. The first step in epidemiological studies of food-borne disease out-

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breaks is finding the answers of some questions (when, where, and who) related to the occurrence of events (Cliver and Riemann, 2002; Simjee, 2007). Furthermore, the environmental investigations are parallel with epidemiological studies in food-borne disease outbreaks that find out how and why the outbreak events occurred based on WHO guideline (WHO, 2008).

Enterotoxigenic *Escherichia coli* (ETEC) is one of the most important agents of travelers' diarrheal diseases in the developing countries that may spread by food and water (Daniels, 2006; Daniels et al., 2000; Qadri et al., 2005). ETEC may be unrecognized from enteric microflora or various gastroenteritis infectious diseases; but they are differentiated from other etiologic agents of gastroenteritis infections in food-borne outbreak events by clinical symptoms and duration of illness (Croxen and Finlay, 2010; Dalton et al., 1999).

According to classic epidemiological theory, which is based on agent, environment, and host roles, the climate variations can impact on all three items by direct extreme events and various indirect effects to distribution, transmission and intensification of the infectious diseases in the emergence of diseases (Wu et al., 2016). Climate variations affect the potential ranges of communicable disease and their transmissions; the weather variations can impact on intensity and frequency of disease outbreak distributions (Epstein, 2002, 2010).

Some studies demonstrated that several climatic parameters such as wind and dust storms were the strong reasons for spreading etiologic agents of infectious disease; therefore the outbreaks maybe distributed from endemic regions to other areas (Wang et al., 2017; Wu et al., 2016; Zhang et al., 2007; Zhang et al., 2010). The main purpose of this study was to determine the association between ETEC outbreaks with climatic and demographic variables in Yazd province of Iran.

Materials and methods

Data collection and sampling

This retrospective cross-sectional study was done on 729 food-borne disease rectal swab samples. As described in our previous study (Ehrampoush et al., 2018), the samples were gathered during 48 ETEC outbreaks from 2012 to 2016, referred to the Food-Borne Disease Research Center, Tehran University of Medical Science, Tehran, Iran. The studied outbreak cases were from different cities of Yazd province, including Yazd (center of Yazd province), Ashkezar, Mehriz, Taft, Meybod, Abarkuh, Khatam, Ardakan, and Behabad (Figure 1; Table 1). All of the patients used contaminated food or water and suffered from vomiting, abdominal cramp, headache, fever, and none bloody diarrhea. The climatic data was gathered from Iran's Meteorological Organization (www.irimo.ir) and Yazd synoptic stations. The daily variable values were calculated monthly, including temperature, relative humidity, rainfalls, and dust term. The geographic coordinates of the cities were extracted from Google Map. The other information related to the demographic parameters of the interested cities were obtained from Statistical Center of Iran, including age, sex, and type of community.

Laboratorial diagnosis

In order to identify the etiologic agents of outbreaks, the rectal swabs of patients were cultured on Lauryl Tryptose broth (17349 Sigma-Aldrich, USA) and incubated for 24-48 h at 35 °C, then transferred to Brilliant Green Bile agar (B1802 Sigma Aldrich, USA) or Eosin-Methylene Blue agar (70186 Sigma-Aldrich, USA) for 18-24 h in 35 °C. Biochemical tests, serotyping, and heat labile enterotoxin assay in Vero cell line culture were carried out for detection of the isolates. For serotyping of the *E. coli* strains, the commercial serotype kit (Baharafshan, Tehran, Iran) was used and ETEC determined by agglutination analysis test (Nazarian et al., 2014; Rodas et al., 2011). The diagnostic test was done at the reference lab of food-borne disease outbreak in School of Public Health, Tehran University of Medical Science, Tehran, Iran.

Statistical analysis

The relationship between the Incidence Rate (IR) of outbreaks with the regional climatic and demographic variables was found by multilevel analysis method for different parts of Yazd province for 2012-2016. The Poisson's regression equation was used to determine the association between ETEC outbreaks IRs and temperature, rainfall, humidity, air suspended dust, type of community, sex and age in different studied cities in Yazd province. For this purpose, the temporal climatic parameters and demographic variables were considered as the independent variable and ETEC food-borne disease outbreak was the only dependent variable in used multilevel regression analysis model (Pearl, 2014; Valcour et al., 2016; Williams et al., 2013).

All affected factors on IR of ETEC outbreaks, including temporal climate parameters and regional demographic variables of Yazd province were analyzed by Stata statistical software (Release 14. College Station, TX: Stata Corp LP). The most likely clusters was identified according to the maximum ratio of log-likelihood, while the $p<0.05$ was considered as significant relationships.

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Results

The parameters of all 91 cases of ETEC food-borne disease recorded in Yazd province during 2012-2016 are indicated in Table 2. As shown in Table 3, IR of ETEC outbreaks in Ashkezar city (4.28), Mehriz city (4.70), and Taft city (4.26) were significantly different from the outbreak IRs in comparison with Yazd city (reference city). No significant (p>0.05) relations were observed between the other cities and IR of ETEC outbreaks. Significant associations were found between some demographic factors and IR of ETEC food-borne outbreak such as age, while the highest IR of disease was in adults with more than 60 years old. There was no relationship between sex and type of community with IR of ETEC food-borne disease (Table 4).

The analysis showed a positive relationship between suspended dust condition and the IR of ETEC outbreaks (Table 5). We found a significant (p<0.05) association between IR of ETEC outbreaks and season. The IR of ETEC outbreak reduced to 75% in winter (IR=0.25, CI: 0.053 to 1.17) in comparison with reference season (spring). However, there was no significant relation (p>0.05) between other seasons and the IR of ETEC outbreak (1.625 for summer and 1.875 for autumn).

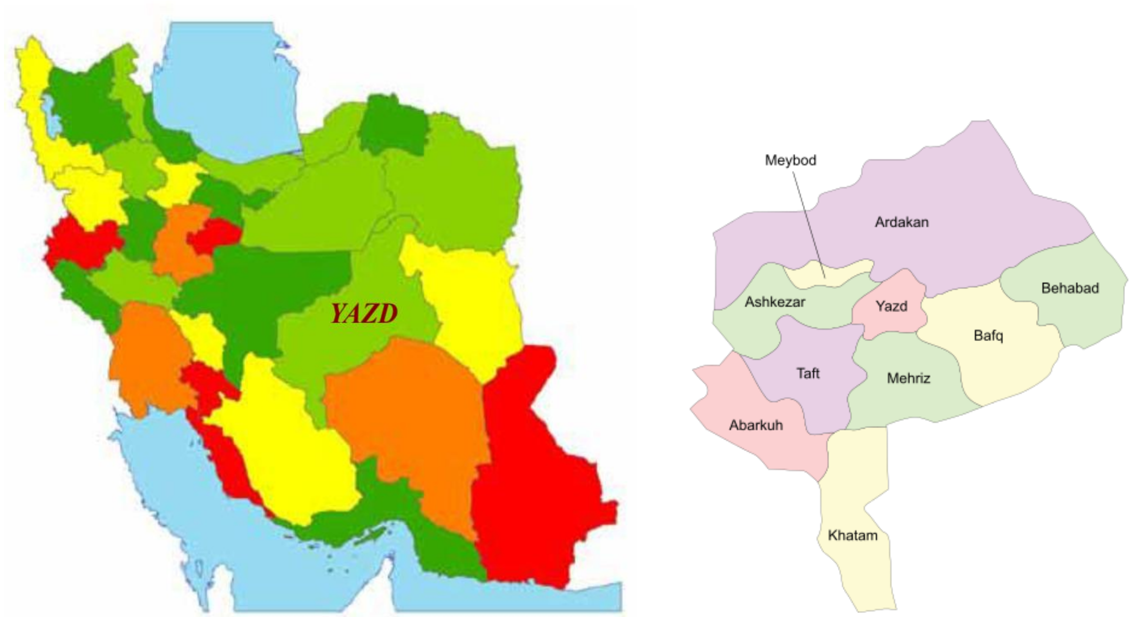

Figure 1: Schematic map of Yazd province, Central Iran

Table 1: Geographic coordinates of different cities' synoptic station in Yazd province, Central Iran

Station	Altitude (m)	Longitude (Degree)	Latitude (Degree)
Abarkuh	1506	53.28	31.13
Ardakan	1104	54.01	32.19
Bafq	950	55.26	31.26
Behabad	1410	56.03	31.51
Khatam	1600	54.04	30.05
Mehriz	1520	54.48	31.57
Meybod	1109	54.01	32.23
Taft	1420	54.06	31.18
Yazd	1230	54.17	31.53

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Table 2: Descriptive outcome of 91 ETEC food-borne outbreaks in Yazd province, Central Iran during 2012-2016

Parameter	Level	Value
Age	Mean±SD	18±18
	Median (range)	11 (2-85)
	≤5	10
	6-20	47
	21-45	25
	46-59	4
	60≤	5
Genus	Female	55
	Male	36
Type of community	Family	53
	Social community	38
Admission	Hospitalized patient	30
	Outpatient	57
	Both	4
Year	2012	11
	2013	14
	2014	25
	2015	38
	2016	3
Season	Spring	17
	Summer	30
	Autumn	34
	Winter	10
Month	Jan	3
	Feb	2
	Mar	6
	Apr	7
	May	9
	Jun	5
	Jul	12
	Aug	0
	Sep	18
	Oct	19
	Nov	4
	Dec	6
Current weather conditions	Normal	30
	Haze	7
	Dust from outside	25
	Dust inside	29
Daily temperature	Mean±SD	25±7.9
	Median (range)	29.5 (7.6-32.4)
Relative daily humidity	Mean±SD	33.4±18.8
	Median (range)	32.5 (6.5-80)
Monthly temperature	Mean±SD	27.9±7.2
	Median (range)	32.7 (9.7-33.4)
Monthly humidity	Mean±SD	22.5±10.1
	Median (range)	20 (12-50)

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Table 3: The incidence rate of ETEC outbreak in different cities of Yazd province, Central Iran

City	IR [a]	*p* value	95%CI [b]	
			Lower	Upper
Yazd	Ref [c]			
Ashkezar	4.28	0.006	1.505	12.177
Mehriz	4.70	0.0001	2.152	10.299
Taft	4.26	0.007	1.499	12.135
Meybod	0.25	0.174	0.341	1.839
Abarkuh	1.39	0.647	0.333	5.857
Khatam	2.74	0.096	0.836	9.018
Ardakan	0.90	0.863	0.274	2.957
Behabad	0.49	0.489	0.673	3.632

a: Incidence Rate of outbreaks; b: Confidence Interval; c: Reference city

Table 4: The relationship of incidence rate of ETEC Food-borne diseases with demographic variables

Demographic variables	Level	No.	IR [a]	95% CI [b]		*p* value
				Lower	Upper	
Genus						
	Female	55	0.75	0.564	0.974	0.178
	Male	36	1.05	0.733	1.45	
Age						
	≤5	10	2.95	1.42	5.43	<0.001
	6-20	47	1.44	1.06	1.92	
	21-45	25	0.60	0.39	0.889	
	46-59	4	4.13	1.13	10.58	
	60≤	5	4.78	1.55	11.15	
Type of community						
	Family	53	0.37	0.278	0.485	0.228
	Social community [c]	38	0.52	0.368	0.713	

a: Incident Rate of disease per100000 populations
b: Confidence Interval
c: Social community indicates the persons who used food of restaurant or live in organizational community

Table 5: The incidence rate of ETEC outbreaks based on climatic parameter

Climatic variables	IR [a]	95%CI [b]		*p* value
		Lower	Upper	
Temperature	0.986	0.872	1.115	0.82
Humidity	0.942	0.218	1.035	0.21
Rainfall	0.984	0.856	1.161	0.85
Air condition				
Normal	Ref [c]			
External dust [d]	2.388	0.923	6.177	0.073
Inner dust [e]	3.911	1.673	9.144	0.002

a: Incident Rate ratio of outbreaks; b: Confidence Interval; c: Reference; d: Dust originated from out of station;
e: Dust originated from inner of station

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Discussion

This research was the first study on the relationship of some environmental conditions and the IR of ETEC food-borne disease outbreaks in Yazd province of Iran identifying the distributions of ETEC outbreaks in various cities of Yazd province. We showed that Mehriz city had the highest contingency of outbreaks among the studied cities that it may be due to its more traveler and tourist attractions than the other cities of Yazd province. Some studies confirmed the role of weak sanitation and contaminated food/water in the transmission of ETEC infections. The fresh fruits and vegetables have also been reported for inducing ETEC food-borne diseases (Ashbolt, 2004; Huerta et al., 2000). It seems that irrigation of vegetable and fruit with contaminated flumes may be an important way of ETEC environmental transmissions in Mehriz city.

Bokhari et al. (2013) showed that ETEC diarrheal disease was the main agent of children outbreaks occurred after consecutive floods in Pakistan during 2010 to 2011. Furthermore, 14 cases of ETEC outbreaks in adults were identified in the United States during 1975 to 1995 affecting 5683 persons (Dalton et al., 1999). The similar research in Iran reported high occurrence of ETEC diarrheal disease (21.9%) in patients with the range age of 3 months to 48 years old in Bandar-Abbas (Katouli et al., 1988). Also, Nazarian et al. (2014) found that 8.04% of Iranian children suffered from the diarrheal disease. Among a population, children less than 5 years and old people are considered as the high risk persons due to their weakness in immunity responses to environmental hazards and challenges (Luber and McGeehin, 2008). We also found significant relationship between age and IR of ETEC food-borne diseases. The most incidence rate of diseases was observed in adults with more than 60 years old which most of them lived in weak sanitation.

The outputs of the present investigation revealed that the IR of ETEC outbreak only increased by inner dust and the number of the patients in autumn was reported in higher rate than other seasons. Due to the role of dust condition in spreading and survive of pathogenic agents in dry weather (Suarez et al., 2008), the increasing rate of outbreaks at autumn was probably associated to this phenomenon in Yazd province. Moreover, the outcomes of multilevel regression analyses confirmed the negative relationship of winter with IR of food-borne disease outbreaks that might be related to the increasing rate of precipitations in winter.

The investigations on traveler diarrhea in Mexico city, capital of Mexico revealed the incidence rate of ETEC outbreaks in summer was more than the other seasons (Flores et al., 2008); however, the ETEC outbreaks in the Yazd province were reported more commonly during the autumn. There are several interventional factors that may influence the pathogen transmission such as sanitation infrastructure, drinking water distribution systems, food handling practices, nutritional habits of societies, etc. (Auld et al., 2004; Newman et al., 2015; Philipsborn et al., 2016; Tarique et al., 2007; Van de Venter, 2000). The earth weather variations may influence the human activities such as seasonal business, immigration, winter-summer habitude, and corporeal exercises that play important roles in human capacities and responses. Accordingly, the seasonal incidence rate of diseases and outbreaks are likely affected by the behaviors and habits of population and their resiliencies (Miraglia et al., 2009; Tirado et al., 2010; Viboud et al., 2004).

Conclusion

This study characterized the ETEC food-borne disease outbreaks in Yazd province of Iran during 2012-2016 and evaluated the relationship between some environmental conditions and food-borne disease outbreaks. We hope that these findings could be useful for planning appropriate health care strategies, increasing the resiliency of communities, preventing and control the local outbreaks. The present work showed the association of ETEC outbreaks with some factors such as demographic features, location status, and climate variations.

Author contributions

M.M.S.D. and M.H.E. designed the project of study; F.A., A.A.D.T., and Z.R. conducted the experiments; M.Y. analyzed the data; F.A. wrote the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

There was no conflict of interest in this study.

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