Research Study

Population-based surveillance of Verocytotoxin-producing *Escherichia coli* in Japan, 1999-2004

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Abstract

The nationwide surveillance system of the National Institute of Infectious Diseases was reengineered to strengthen the ability to determine the number of laboratory-confirmed verocytotoxin-producing *Escherichia coli* (VTEC) cases across the nation as of April 1999. Nationwide, 19,304 cases of laboratory-confirmed VTEC were reported between April 1999 and October 2004. The annual incidence was 2.74 per 100,000 populations. After adjusting for climate factors, VTEC incidence rates were significantly higher in prefectures with a 1) higher percentage of elderly people; 2) higher population density; 3) higher number of people in a household; and 4) higher number of beef cattle per population. In conclusion, by conducting nationwide surveillance in Japan, the high annual incidence of VTEC was implied to be associated with climate, socioeconomic, and population factors. However, these associations were based upon an ecological study and further longitudinal study is obligatory to address these complicated associations as future directions.

Introduction

Since the early 1980s, it has been known that Verocytotoxin-producing *Escherichia coli* (*E. coli*) (VTEC) induces hemorrhagic enterocolitis in humans and is associated with a wide range of clinical illnesses, including non-bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and death (1). Verocytotoxin is considered to trigger serious condition in patients infected with the pathogenic *E. coli* (1). *E. coli* O157 and other serotype of pathogenic *E. coli* were found regularly in the feces of healthy cattle (2), and are considered to transmit to humans through contaminated food (3), water (4), and direct contact with infected people (5) or animals (6). Thus, VTEC infections are prevalent among countries that raise cattle, including Japan (7), the United States (8), Canada (9), England (10), Scotland (11), Argentina (12), Australia (13), and New Zealand (14).

Triggered by two major outbreaks of VTEC in Japan (4, 15), the nationwide surveillance system of the National Institute of Infectious Diseases was reengineered in April 1999 to better ascertain the state of laboratory-confirmed VTEC cases across the nation by enacting the new Infectious Disease Prevention Law. This system enabled us to conduct a population-based surveillance study of the incidence of VTEC. In this ecological study, we used this nationwide surveillance data to explore factors that affect the incidence of VTEC.

Methods

VTEC Surveillance Data

Data on cases of VTEC in humans reported in Japan from April 1999 to October 2004 (287 weeks) were retrieved from the Infectious Agents Surveillance Report published by the National Institute of Infectious Diseases in Japan.

When the clinician suspects hemorrhagic enterocolitis due to pathogenic *E. coli* for the patients judging from clinical symptoms such as hemorrhagic colitis, the stool samples or rectal swabs obtained from these patients are sent to laboratories at the hospital, private bio-companies for clinical diagnosis, national institutions in each prefecture or the National Institute of Infectious Diseases. To keep sensitivity and specificity in detecting VTEC high quality, the protocol and trained skills in these laboratories are under the guidance of National Institute of Infectious Diseases. At these laboratories, the specimens are cultured on specific media such as CHROMagar O157 (CHROMagar, Paris, France) or cefixime-tellurite sorbitol MacConkey (CT-SMAC) agar (Oxoid, Unipath Ltd., Hampshire, UK) (16, 17) and specific antibodies against each serotype of *E. coli* are employed (16). If the existence of pathogenic E. *coli* is confirmed, the ability for production of Verocytotoxin was next investigated from isolates by using the method of reversed passive latex agglutination (VTEC-RPLA) and/or of a multiplex PCR assay (16, 18, 19).

If the production of Verocytotoxin was confirmed by laboratory bases, the case was considered as a symptomatic VTEC. Depending on the doctor's discretion, people around the case may be further examined for VTEC. When the doctor diagnosed VTEC infection either symptomatic or asymptomatic, he or she is on duty to report this event to the local health center immediately and deal with the cases to prevent further spread of the disease, which is according to new Infectious Disease Prevention Law. Then, the number of cases with VTEC in Japan are counted and totaled for each prefecture on a weekly basis.

Climate variables

We obtained weekly summarized data for a variety of climate parameters from Meteorological Agencies in the capitals of the 47 prefectures. These parameters included average air temperature of the day (ºC: mean=16.1, range: -7.5 to 31.2), relative humidity (%: mean=68.8, range: 34.6 to 93.3), sea level atmospheric pressure (hPa: mean=1014.1, range: 995.1 to 1028.6), vapor pressure (hPa: mean=14.4, range: 2.2 to 32.6), wind speed (m/s: mean=2.9, range: 0.9 to 10.3), cloud cover (mean=6.9, range: 0 to 10), number of sunny days (days mean=6, range: 1 to 7), and amount of precipitation (mm: mean=31.7, range: 0 to 704.5). We used data on climatic conditions from the 2-week period prior to when each case of VTEC was reported to approximate the incubation period between infection and reporting a diagnosis of VTEC.

Socioeconomic data

We retrieved annual social and economical data in a total of 47 prefectures in Japan (20), including the following information: population density; percentage of children (aged 15 and younger); percentage of elderly (aged 65 and older); the average number of people in the household; the number of livestock (beef cattle plus dairy cows, pork, and chickens) per population in the prefecture; and the average income.

Statistical analyses

In each prefecture, variables associated with the incidence of VTEC per 100,000 populations during a week were analyzed with multiple linear regression models using climate and socioeconomic variables. All statistical analyses were performed using STATA 8.0 software (STATA Corporation, College Station, TX).

Results

Incidence of VTEC

Nationwide, 19,304 cases of VTEC were reported between April 1999 and October 2004. The annual incidence was 2.74 per 100,000 populations. The highest number that occurred in a prefecture was 63 VTEC cases/1,000,000 populations during a week in a single prefecture. More than 16 VTEC cases were observed in 10% of a total of 13,489 weeks (287 weeks times 47 prefectures). On the other hand, no cases were reported in 57.6% of the 13,489 weeks. Age distribution of patients with VTEC was shown (Fig. 1). The number of VTEC cases was the highest in age group less than 5 years old. In the older age groups, less number of cases was reported. Totally 65 outbreaks defined as more than 11 laboratory-confirmed VTEC cases in a certain time frame and local area were reported during the study period as shown in Table. 1. The biggest outbreak occurred at September 2003 in Kanagawa prefecture: 252 symptomatic VTEC/197 asymptomatic VTEC. The next was at March 2001 in Chiba prefecture: 195 symptomatic VTEC/62 asymptomatic VTEC.

Ecological studies of VTEC incidence

Data showed an annual oscillation of VTEC cases during the study period (Fig. 2). The temporal distribution of VTEC cases showed a marked seasonal oscillation pattern with peaks centered in July and August. The average air temperature of a week (≧25℃) during the summer season was overlaid in the graph. In 2003, we experienced a cool summer, during which the number of cases of VTEC was noted to be lower than in other years. The annual incidence showed no clear tendency to increase or decrease during the study period.

Geographic distribution of VTEC cases/100,000 population per year in 47 prefectures is shown in Fig. 3. A relatively higher incidence of VTEC was clustered in some western sections of several Japanese prefectures, including Saga, Nagasaki, Fukuoka, Miyazaki, Tottori, Shimane, Okayama, Shiga, Fukui, Ishikawa, and Kanazawa, and some northeastern sections of two Japanese prefectures, including Iwate and Akita. The four prefectures with the highest annual incidences were located in rural areas: Saga (9.2/100,000), Ishikawa (7.9/100,000), Akita (5.8/100,000), and Iwate (5.8/100,000), whereas the prefectures with the lowest incidences were near urban areas: Yamanashi (1.3/100,000), Ibaraki (1.1/100,000), Niigata (0.9/100,000), and Shizuoka (1.4/100,000).

Variables associated VTEC

Three of the eight climate variables—average air temperature of the day (t = 6.03), wind speed (t = 7.16), and the number of sunny days (t = 4.01)—were significantly associated ($P < 0.001$) with of the incidence of VTEC cases/100,000 population per week per prefecture under multiple linear regression analysis (Table 2).

By adjusting for these 8 climate variables as well as calendar months, associations of 7 socioeconomic variables with VTEC incidence/100,000 population per week per prefecture were analyzed with multiple regression analysis (Table 3). The strongest risk factor for VTEC occurrence in the model was a higher percentage of elderly people in the prefecture (t = 20.77; $P < 0.001$). Although the presence of children in the prefecture was a risk factor, the association was weak (t = 2.35; P = 0.019). Higher population density (t = 8.81; $P < 0.001$) and the average number of people in a household of the prefecture (t = 5.98; $P < 0.001$) were positively associated with VTEC incidence. On the other hand, average income in the prefecture showed a negative association with VTEC incidence (t = –10.37; $P < 0.001$). The number of beef cattle per population in the prefecture was positively associated with VTEC incidence ($t = 2.35$; $P = 0.019$); in contrast, the number of chickens per population was negatively associated with VTEC incidence ($t = –3.41$; $P = 0.001$).

The expected number of VTEC cases based on this multiple regression model was compared with the observed number of cases (Fig. 4). Due to strong collinearly with beef cattle, the variable of pork was not used in this modeling. The correlation was 0.56; 32% of the variability could be explained by these socioeconomic and climate factors.

Discussion

Based on national surveillance data in Japan, the annual incidence of laboratory-confirmed VTEC was calculated as 2.74/100,000 population. In England and Wales, population-based surveillance studies for VTEC showed an annual incidence of 1.28-2.10/100,000 population during the 4-year period from 1995 to 1999 (21, 22). Similarly, the number of cases in other countries was as follows: Spain (0.01/100,000); Italy (0.02/100,000); Netherlands (0.06/100,000); Finland (0.1/100,000); Denmark (0.12/100,000); Austria (0.14/100,000); Germany (0.4/100,000); Belgium (0.52/100,000); Sweden (1.36/100,000) (23). In the United States, 3,674 cases were reported annually to the surveillance system (24). Thus, compared with surveillance data in other developed countries, the incidence in Japan seems to be relatively high. However, we can not compare the annual incidence among countries exactly, since the surveillance system is different in each country. Moreover, we compared surveillance data in different geographic regions of Japan by assuming 1) people seek care with the same frequency in all regions when they are ill 2) doctors request stool specimens with the same frequency in all regions 3) laboratories test for VTEC and report VTEC with same frequency in all regions. Thus, some degree of observation bias may exist even under control of the law, which is a limitation of this study. In addition, number of cases is including not only symptomatic VTEC but also asymptomatic VTEC, which may also raise the incidence of VTEC in Japanese surveillance system.

The seasonal oscillation pattern of VTEC, that is, high in summer and low in winter, was similar to other ecological studies (25, 26). Considering that shedding from animals is also seasonal (27) and that food-borne disease tends to be prevalent in the summer (28), climatic factors play an important part in determining the incidence of VTEC. Some of climate variables were associated with the occurrence of VTEC, which is not beyond the hypothesis yet and need to prove by further investigation.

Beef cattle and dairy cows, but not chicken, per population in the prefecture was positively associated with incidence of VTEC. In Japan, the prevalence of VTEC isolated from fecal samples was 5% to 10% (29), as high as the United States and Sweden, and higher than Poland and other countries (30). Shedding of VTEC from infected cattle persists for <1 week to 10 weeks (31), and VTEC strains can be transferred with considerable frequency over global distances (32). Moreover, the correlation between variables of the agricultural structure and human VTEC incidence has been shown to be high based on geographical information (26). Our results appear to be consistent with these previous studies.

Although this is an ecological study, there was a relationship between population factors and VTEC incidence. It was demonstrated that higher population density and more than the average number of people per household in the prefecture were positively associated with the incidence of VTEC. Population density and the number of people per household may enhance the chance of contact between sensitive person-to-person spreading of VTEC with or without symptoms. This assumption is not inconsistent with classical mathematical models of population dynamics for infectious diseases transmitted by pathway of person-to-person (33).

In conclusion, by conducting nationwide surveillance in Japan, we showed a high annual incidence of VTEC 2.74/100,000 population that was associated with climate, socioeconomic, and population factors. However, these associations were based upon an ecological study and further longitudinal study is obligatory to address these complicated associations as future directions.

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Table 1. Outbreaks[*1] of infection with Verocytotoxin-producing Escherichia *coli* (VTEC) in Japan, 1999-2004.

No.	Year. Month	Prefecture	Setting	Serotype	VT type[*2]	The number of patients with symptoms	The number of positive cases	The number of test subjects
1	1999.7	Hokkaido	Nursery	O26 : HNT	VT 2	NR[*3]	64	NR
2	1999.7	Nagano	Home	O157 : H7	VT 1&2	NR	21	NR
3	1999.8	Hyogo	Nursery	O157 : H7	VT 2	NR	12	NR
4	1999.9	Osaka	Nursery	O26 : H11	VT 1	NR	11	NR
5	1999.9	Chiba	Nursery	O26 : H11	VT 1	NR	13	NR
6	1999.10	Nagasaki	Nursery	O26 : H11	VT 1	NR	17	NR
7	1999.11	Fukuoka	Nursery	O111 : H-	VT 1	NR	16	NR
8	2000.5	Toyama	Hospital	O157 : H7	VT 1&2	NR	15	743
9	2000.6	Kanagawa	Health facilities for the aged	O157 : H7	VT 2	56	87	842
10	2000.6	Ishikawa	Nursery	O26 : H11	VT 1	1	16	180
11	2000.6	Osaka	Nursery	O26 : H11	VT 1	2	20	211

12	2000.8	Toyama	Nursery	O111 : H-	VT 1	2	14	240
13	2000.8	Osaka	Camp	O157 : H7	VT 2	2	11	59
14	2000.8	Shimane	Unknown	O26 : H-	VT 1	7	11	NR
15	2000.9	Fukuoka	Nursery	O26 : H11	VT 1	8	33	251
16	2000.10	Chiba	Event place	O157 : H7	VT 2	41	58	1304
17	2000.11	Shizuoka	Unknown	O26 : H11	VT 1	2	19	355
18	2001.2	Kyoto	Nursery	O26 : HNT	VT 1	5	17	256
19	2001.3	Chiba	Home	O157 : H7	VT 1&2	195	257	NR
20	2001.4	Osaka	Nursery	O157 : H7	VT 1&2	13	27	236
21	2001.5	Ishikawa	Dormitory	O157 : H7	VT 2	18	15	261
22	2001.6	Shimane	Home	O26 : H11	VT 1	1	11	125
23	2001.7	Osaka	Nursery	O26 : H11	VT 1	17	30	231
24	2001.7	Ehime	Hospital	O157 : HNT	VT 1&2	6	26	1382
25	2001.7	Fukushima	Unknown	O157 : H7	VT 2	4	15	92
26	2001.7	Nara	Nursery	O157 : H7	VT 1&2	21	21	244

27	2001.8	Shimane	Nursery	O26 : H-	VT 1	6	28	206
28	2001.8	Akita	Nursing Home	O157 : H7	VT 1&2	5	16	220
29	2001.8	Saga	Nursery	O26 : HNT	VT 1	3	33	142
30	2001.8	Fukuoka	Nursery	O26 : H11	VT 1	NR	44	830
31	2001.8	Nagano	Restaurant	O157 : H7	VT 1&2	29	22	525
32	2001.8	Saitama	Nursing Home	O157 : H7	VT 1&2	26	67	260
33	2002.4	Saga	Nursery	O121 : H19	VT 2	16	23	163
34	2002.4	Ishikawa	Kindergarten	O157 : H7	VT 2	5	12	303
35	2002.4	Hyogo	Restaurant	O157 : H7	VT 1&2	30	52	NR
36	2002.6	Saga	Nursery	O111: H-	VT 1	10	47	519
37	2002.6	Fukuoka	Nursery	O157 : H-	VT 2	74	112	542
38	2002.7	Iwate	Elementary school	O111: H-	VT 1	4	15	331
39	2002.7	Hokkaido	Nursery	O26 : 11	VT 1	21	54	429
40	2002.7	Nagano	Elementary school	O26 : H11	VT 1	45	55	11889
41	2002.7	Miyagi	Nursery	O26 : H11	VT 1	12	21	167

42	2002.7	Iwate	University	O26 : HNT	VT 1	NR	23	NR
43	2002.8	Tochigi	Health facilities for the aged	O157 : H7	VT 1&2	123	111	1521
44	2002.8	Hyogo	Health facilities for the aged	O157 : H7	VT 1&2	NR	13	274
45	2002.8	Osaka	Nursery	O26 : HUT	VT 1	13	23	153
46	2002.9	Ishikawa	Nursery	O157 : H7	VT 1&2	18	36	607
47	2003.5	Gifu	Dormitory	O157 : H7	VT 2	6	15	1247
48	2003.5	Gifu	Nursery	O157 : H-	VT 1&2	11	43	300
49	2003.7	Fukui/Kyoto	Restaurant	O157 : H7	VT 1&2	8	11	NR
50	2003.7	Hyogo	Nursery	O26 : H11	VT 1	18	16	>195
51	2003.8	Fukuoka	Home	O157 : H7	VT 1&2	11	16	NR
52	2003.8	Ishikawa	Nursery	O26 : H11	VT 1	18	29	238
53	2003.9	Chiba	Nursery	O103 : H2	VT 1	13	13	119
54	2003.9	Kanagawa	Kindergarten	O26 : H11	VT 1	252	449	6037
55	2003.11	Kyoto	Nursery	O157 : H7	VT 1&2	37	60	396

56	2003.12	Fukuoka	High school	O157 : H7	VT 1&2	16	61	967
57	2004.5	Ehime	Kindergarten	O26 : H11	VT 1	NR	38	460
58	2004.6	Chiba	Elementary School	O121 : H19	VT 1	63	17	121
				O157 : H7	VT 1&2	0	2	121
59	2004.6	Hyogo	Nursery	O26 : H11	VT 1	2	11	>149
60	2004.7	Miyagi	Nursery	O26 : H11	VT 1	NR	12	119
61	2004.7	Ishikawa	High school	O111 : H-	VT 1&2	110	103	715
62	2004.7	Miyagi	Nursery	O26 : H11	VT 1	9	23	188
63	2004.8	Kanagawa	Nursery	O157 : H7	VT 2	2	17	276
64	2004.8	Ehime	Nursery	O26 : H11	VT 1	NR	15	416
65	2004.8	Mie	Nursery	O157 : H7	VT 2	18	23	278

*1: outbreaks defined as more than 11 laboratory-confirmed VTEC cases in a certain time frame and local area

*2: VT type was detected by using VTEC –RPLA or PCR methods.

*3: NR: Not recorded

Table 2. Climate variables associated with the number of cases of Verocytotoxin-producing Escherichia *coli* (VTEC)[*1] under multiple linear regression[*2]

Climate variable[*3]	t	*P* value
Average air temperature of the day (ºC)	6.03	< 0.001
Relative humidity (%)	1.64	NS
Wind speed (m/s)	7.16	< 0.001
Sea level atmospheric pressure (hPa)	–0.89	NS
Vapor pressure (hPa)	–1.23	NS
Cloud cover	–1.70	NS
Number of sunny days	4.01	< 0.001
Amount of precipitation (mm)	1.18	NS

*1: 5,768 of 13,489 weeks (287 weeks times 47 prefectures) or 42.8% of the weeks were included in the analysis as no cases of EHEC were reported during 7,721 weeks (57.6%)

*2: $R^2 = 0.12$

*3: Data for the 2-week period prior to the week EHEC was reported was used to approximate the period between infection and diagnosis.

Table 3. Socioeconomic variables associated with the number of cases of Verocytotoxin-producing Esch*erichia coli* (VTEC)[*1] under multiple linear regression[*2]

Socioeconomic variable[*3]	t	*P* value
Average number of people in a household[*5]	5.98	< 0.001
Population density	8.81	< 0.001
Percentage of children (15 years and younger)	2.35	0.019
Percentage of old (65 years and younger)	20.77	< 0.001
Average income[*4]	-10.37	< 0.001
Beef cattle / population[*6]	2.35	0.019
Chicken / population	-3.41	0.001

*1: 5,768 of 13,489 weeks (287 weeks times 47 prefectures) or 42.8% of the weeks were included in the analysis as no cases of EHEC were reported during 7,721 weeks (57.6%)

*2: R^2 = 0.32: Calculated based on the multiple linear regression model using the seven variables listed above plus eight climate variables and calendar months.

*3: Annual data in each prefecture were used.

*4: Correlation between average income and population density, average number of people in a household, and percentage of elderly was 0.9, –0.4, and –0.6, respectively.

*5: Correlation between average number of people in a household and population density was –0.4.

*6: Beef cattle / population had a strong correlation with pork / population.

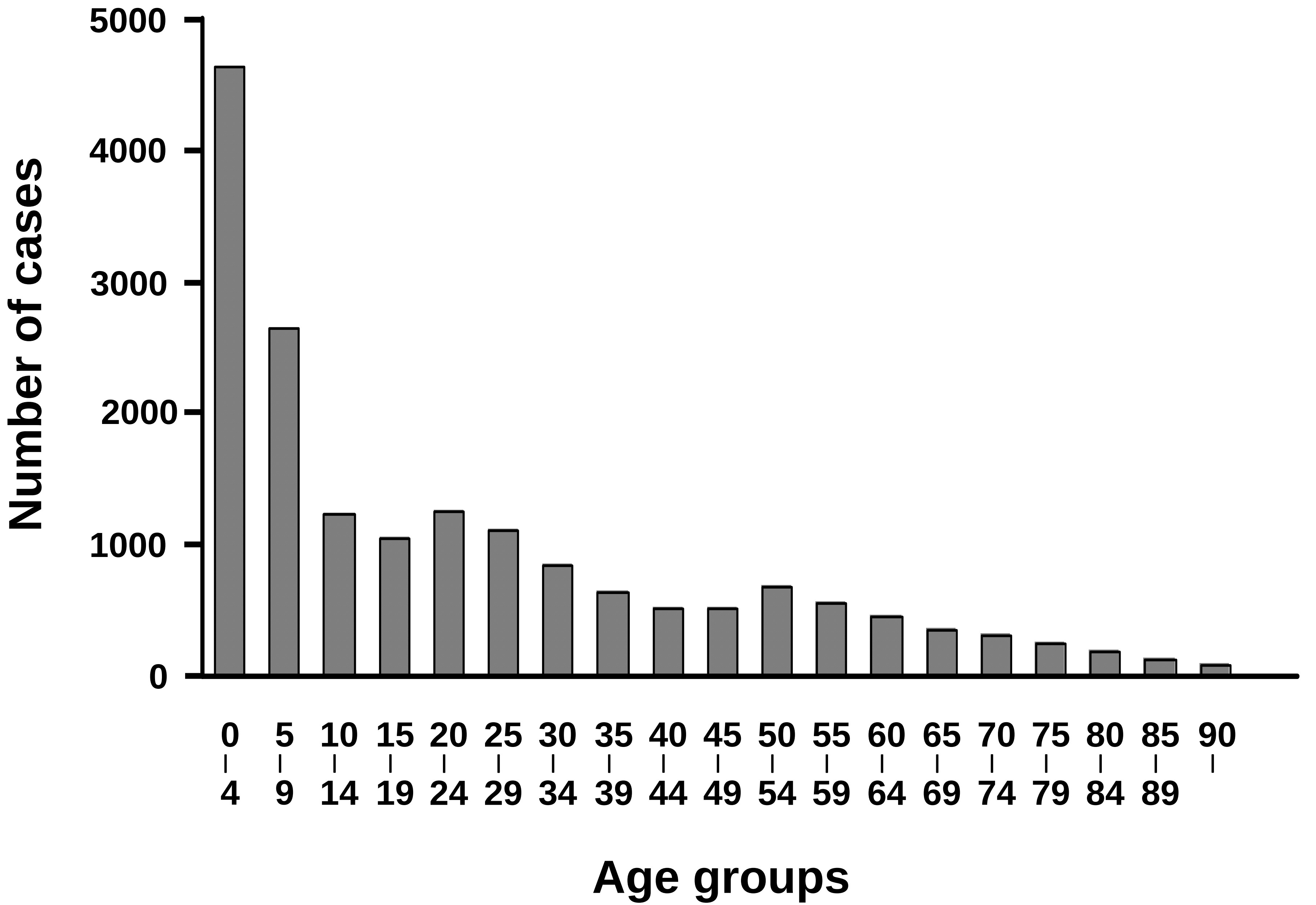
Figure legends

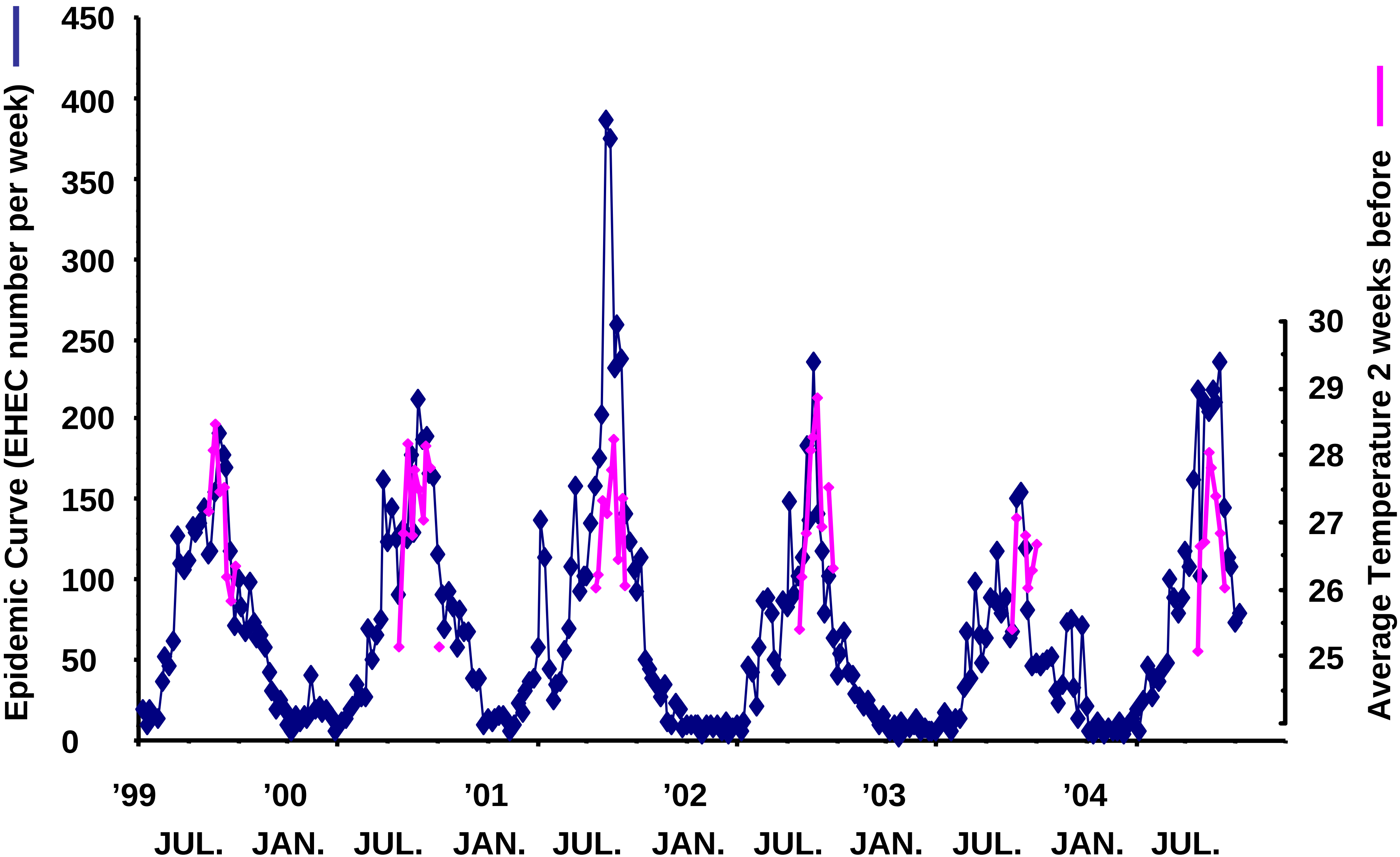
Figure 1. The age distribution of the VTEC cases.

Figure 2. The annual oscillation of Verocytotoxin-producing *Escherichia coli* (VTEC) cases during the study period. In addition to the VTEC cases, the average air temperature of a week (>=25℃) during the summer season was overlaid in the graph.

Figure 3. Geographic distribution of the average number of VTEC cases per 100,000 per year in each 47 prefectures during 1999-2004.

Figure 4. The association between observed number of VTEC cases (Y axis) and expected number of VTEC cases (X axis) computed by a multiple linear regression model using 8 climate variables as well as calendar months and 7 socioeconomic variables


Number of cases
5000
4000
3000
2000
1000
0
0-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
40-44
45-49
50-54
55-59
60-64
65-69
70-74
75-79
80-84
85-89
90-
Age groups



Epidemic Curve (EHEC number per week)
Average Temperature 2 weeks before
0
50
100
150
200
250
300
350
400
450
25
26
27
28
29
30
'99
'00
'01
'02
'03
'04
JUL.
JAN.
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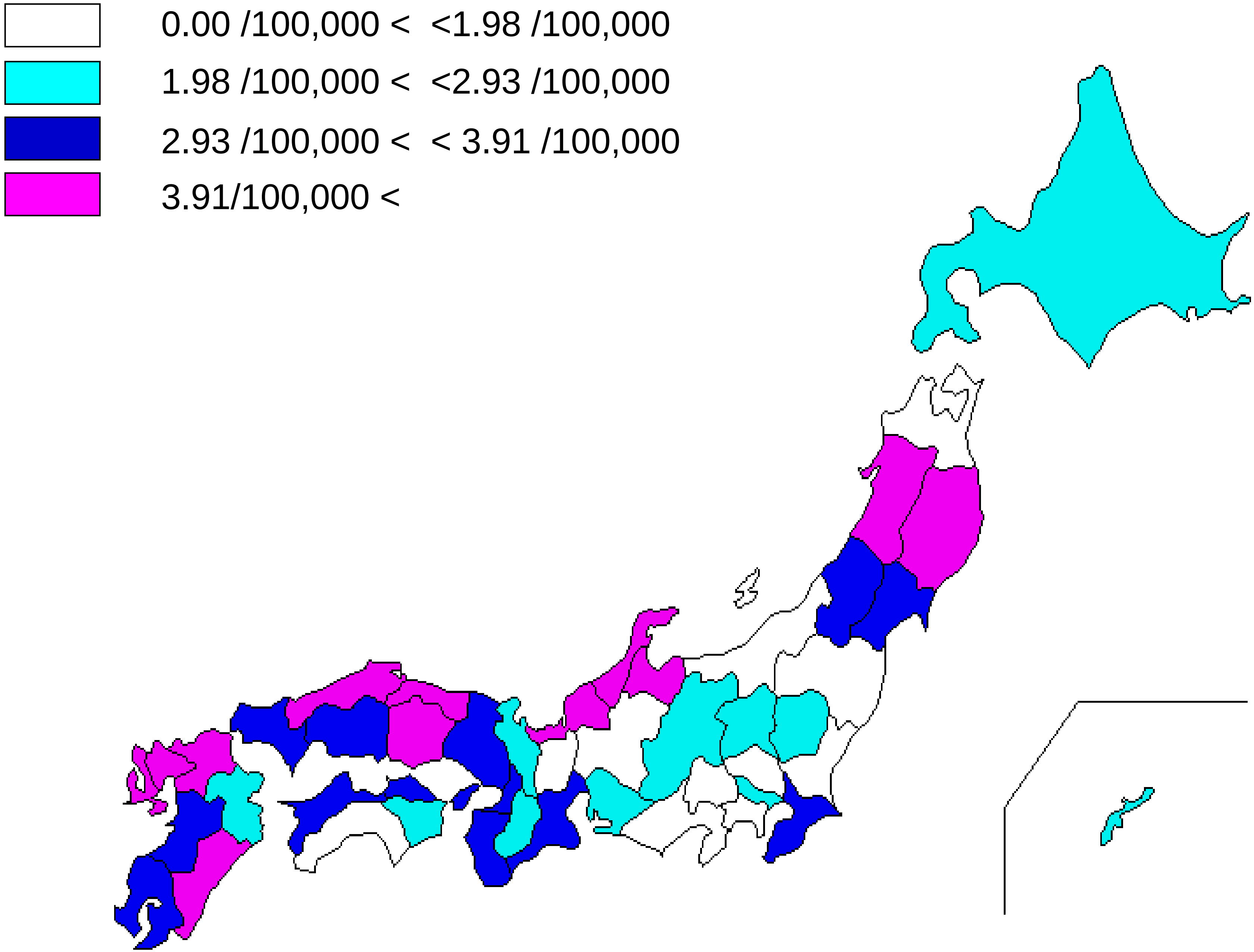
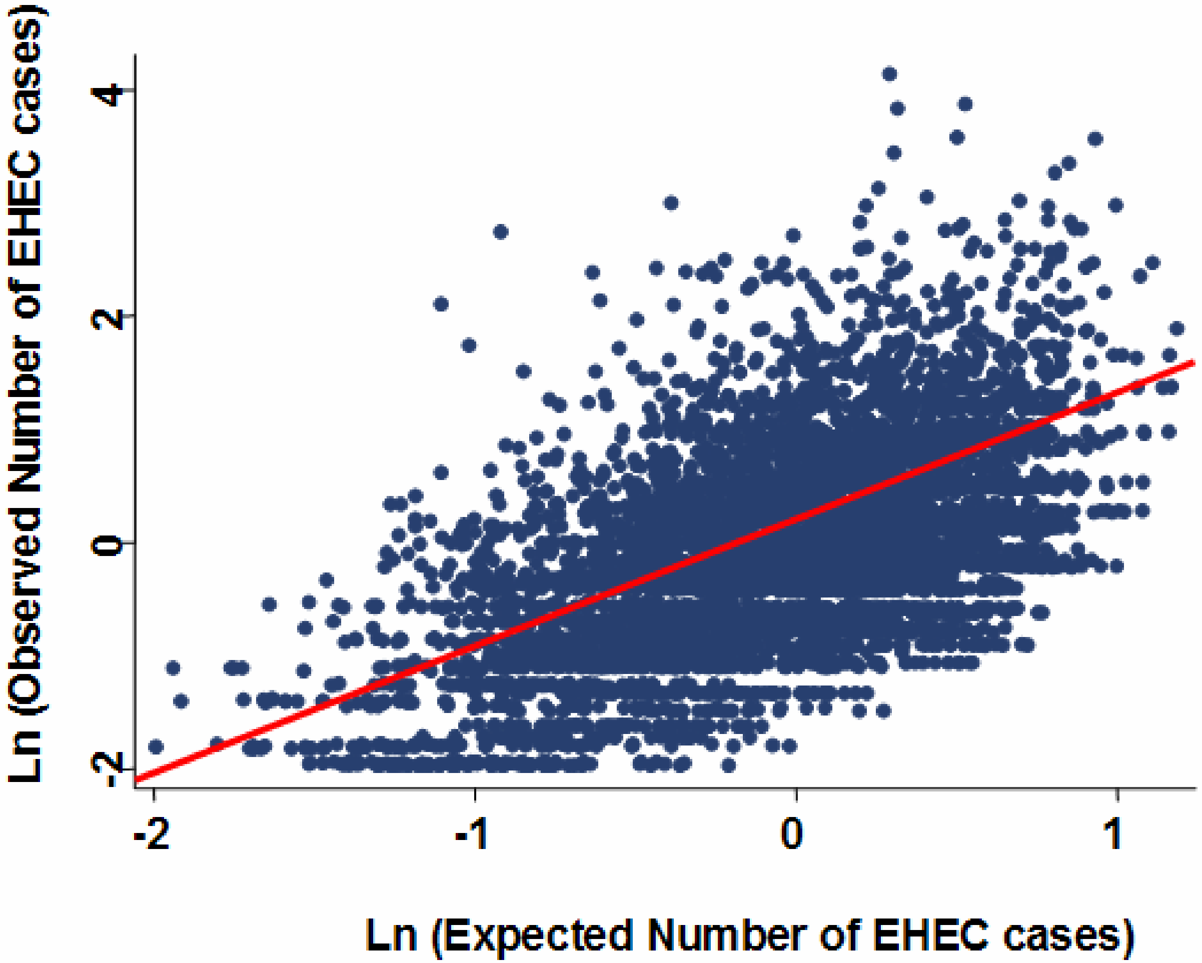


0.00 /100,000 < <1.98 /100,000
1.98 /100,000 < <2.93 /100,000
2.93 /100,000 < < 3.91 /100,000
3.91/100,000 <



Ln (Observed Number of EHEC cases)
4
2
0
-2
-2
-1
0
1
Ln (Expected Number of EHEC cases)