Preplanned Studies

Enterohemorrhagic *Escherichia coli* O157:H7 — Xuzhou City, Jiangsu Province, China, 2001–2021

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Summary

What is already known about this topic?

The largest and longest outbreak of diarrhea, which was complicated with hemolytic uremic syndrome (HUS) caused by enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, occurred in Xuzhou City and its adjacent areas from 1999 to 2000 in China.

What is added by this report?

According to surveillance results from 2001 to 2021, there was a significant decrease in the isolation rate of O157:H7, and cattle and sheep remained the primary hosts. However, non-Shiga toxin-producing O157:H7 emerged as the dominant strain, with *stx2+stx1-* strains following closely behind.

What are the implications for public health practice?

National surveillance of O157:H7 effectively serves as an early warning system and guidance for assessing the intensity and trend of disease epidemics. It is crucial to raise awareness of the public health risks associated with Shiga toxin-producing *E. coli*.

Escherichia coli (*E. coli*) that produce Shiga toxin or verocytotoxin is commonly known as Shiga toxin-producing *E. coli* (STEC) or verocytotoxin-producing *E. coli* (VTEC). Hemorrhagic enteritis (HC), caused by foodborne zoonotic pathogen enterohemorrhagic *E. coli* (EHEC) O157:H7, was first reported in the United States in 1982 and has since caused outbreaks of human infections in various countries such as Canada, Japan, and China. In China, the pathogen was isolated from the feces of patients with diarrhea in Xuzhou City, Jiangsu Province in 1986. It later caused an outbreak in Xuzhou and adjacent areas from 1999 to 2000 (*1*). Measures that included investigation of diarrhea patients, identification of the pathogen, and clearing epidemiological characteristics were implemented to control the spread of the disease. Subsequently, a surveillance program was reformulated in 2001 in Tongshan District, Xuzhou City based on

the national O157:H7 surveillance program and the reality of Jiangsu Province. The program included 18 towns for diarrhea patients and food surveillance, and 3 major animal farming towns for animal surveillance. The program has led to changes in the detection rates and virulence genes of the strains. In this study, we analyzed O157:H7 surveillance data from 2001 to 2021 to predict possible transmission risks.

Between 2001 and 2021 (April to October), raw and cooked meat samples, as well as fecal samples from patients with diarrhea, cattle, sheep, chickens, and pigs were collected by medical workers from local township health centers under the guidance of Tongshan District Center for Disease Control and Prevention during the epidemic season. Isolation of the samples was performed using immunomagnetic bead adsorption. The isolated strains were analyzed via polymerase chain reactions (PCR) detection of virulence genes, namely *stx*, *eaeA*, and *hly*, to determine their virulence characteristics. During the outbreak period from 1999 to 2000, samples were predominantly collected from epidemic villages and towns. Correlation analysis of O157:H7 isolation rates between host animals, diarrhea patients and meat samples from 2001 to 2021 was performed via SPSS (version 19.0, IBM Corp, NY, USA). $P<0.05$ was considered statistically significant.

The 20-year surveillance period, from 2001 to 2021, revealed that the isolation rates of animal feces, meats, and diarrhea patients were 0.41% (31/7,539), 0.12% (3/2,526), and 0.07% (4/5,491), respectively. A pairwise correlation analysis was conducted on the O157:H7 isolation rates from 2001 to 2021 among the aforementioned samples, which found a significant correlation between diarrhea patients and meat, with a correlation coefficient of r=0.745 ($P<0.001$). No significant correlation was found between the isolation rate of host animals and either diarrhea patients or meat. During the O157:H7 outbreak from 1999 to 2000, the isolation rates were 13.56% (154/1,136) in the feces of host animals, 4.35% (7/161) in meat, and 3.88% (52/1,339) in the feces of diarrhea patients.

Compared to the outbreak period, the isolation rates of O157:H7 from various types of samples were significantly reduced after surveillance sites were established in 2001. After 2011, no O157:H7 strain was isolated from diarrhea patients (Figure 1).

Between 1999 and 2000, there was an outbreak period, during which the isolation rates of O157:H7 were observed from cattle, sheep, chickens, and pigs. The isolation rates were found to be 19.51% (8/41), 19.06% (57/299), 10.97% (34/310), and 13.13% (39/297), respectively. Cattle and sheep were identified as the most significant carriers of O157:H7 during this outbreak.

However, surveillance results from 2009 to 2021 showed that the isolation rates of feces from cattle, sheep, and pigs were substantially reduced to 1.05% (11/1,049), 0.55% (6/1,100), and 0.21% (2/963) respectively. Notably, O157:H7 was no longer isolated from chicken feces. Even with this decrease in isolation rates, cattle and sheep remain the primary carrier hosts

of O157:H7.

Shiga toxin is a crucial virulence factor for O157:H7 and is strongly linked to severe complications such as hemolytic uremic syndrome (HUS) (*2*). We isolated 22 strains of O157:H7 between 2009 and 2021, all of which tested positive for *eaeA* and *hly* genes. The gene profiles for Shiga toxin were *stx2+ stx1-* in 22.73% (5/22) of the strains, *stx2+ stx1+* in 4.54% (1/22), and *stx2- stx1-* in 72.73% (16/22), indicating that non-Shiga toxin-producing EHEC O157:H7 strains have become dominant (Table 1).

DISCUSSION

Following the outbreak of O157:H7 in 1999, Xuzhou launched a comprehensive patriotic health campaign focusing on managing water, diet, feces, and eliminating flies. This led to timely control of the disease spread, and a national surveillance site was
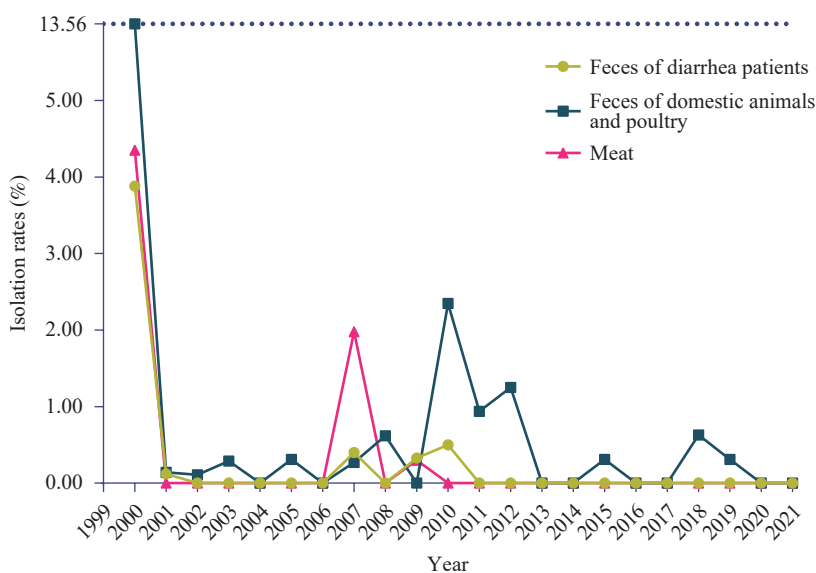

FIGURE 1. Variation curve of isolation rates of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 from various samples, 1999–2021.

TABLE 1. The distribution of virulence genes of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 from different sources of samples, 2009–2021.

Source of sampling	No.of detected samples	No.of positive samples	*stx2+ stx1- eaeA+ hly+*	*stx2+ stx1+ eaeA+ hly+*	*stx2- stx1- eaeA+ hly+*	Positive rate (%)
Diarrhea patients	3,240	2	1	0	1	0.06
Cattle	1,049	11	2	1	8	1.05
Sheep	1,100	6	1	0	5	0.55
Pigs	963	2	0	0	2	0.21
Meat	1,026	1	1	0	0	0.10
Total	7,378	22	5	1	16	0.30

established in Tongshan District which was the epicenter of the outbreak. From 2001 to 2021, surveillance reports showed a significant reduction in the incidence of infectious diarrhea caused by O157:H7 and the host carrier rates. Since 2011, no cases of O157:H7 infection were found in diarrhea patients. However, the pathogen was still prevalent in the feces of animal hosts, with cattle and sheep being the main carriers. Therefore, it is necessary to enhance health education among farmers, especially among individual free-range households, to regulate the proper disposal of host animal fecal waste. This action is essential to prevent the spread and infection of O157:H7.

In Xuzhou, sporadic cases of human infection with O157:H7 have been reported, but there have been no reports of clusters in recent years. This may be due to improved health education and hygiene practices, as well as a decrease in the rate of O157:H7 infection among diarrheal patients, which may be related to a decrease in the carrier rate among host animals and changes in the virulence gene profiles of strains, reducing their pathogenicity. EHEC O157:H7 can produce Stx1 or Stx2 Shiga toxins, with Stx2 more often associated with human disease than Stx1 (*3*). Strains that produce only Stx2 are considered to be more virulent than strains that produce both Stx2 and Stx1, or only Stx1 (*4*). During 1999–2000, *stx2+ stx1-* O157:H7 strains predominated in the region, which may have been the main cause of the serious outbreak (*5*).

Surveillance from 2009–2021 found that Shiga toxin-free (*stx2- stx1-*) strains are now dominant, while *stx2+ stx1- eaeA+ hly+* strains remain the main producers of Shiga toxins in the region. Loss of *stx*-encoding bacteriophages may occur during infection or culturing of strains, resulting in O157:H7 strains lacking *stx* virulence factors (*6*). Therefore, it is important to remain vigilant of changes in the O157:H7 virulence gene profile and improve our ability to identify outbreak risks early.

Limitations of this study include missing data on the virulence profile of strains from 2001 to 2008 due to improper preservation, and an incomplete understanding of the virulence trend.

The United States was the first country to identify O157:H7 as a foodborne pathogen that caused outbreaks. Recently, according to the US CDC (www.cdc.gov/ecli/outbreaks.html), outbreaks of O157:H7 infection have mostly been associated with the consumption of green vegetables such as spinach and lettuce, whereas in the past, the main cause was related to the consumption of beef and related products (*7*). During 1999–2000, there was an O157:H7 outbreak in Xuzhou, which was mainly caused by water and food contaminated with human and animal feces. Host animals and infected persons were the primary sources of infection (*8*). As China's national economic level improves, people's living habits are changing, and there is an increase in the number of people who eat green organic vegetables, especially raw food. Green organic vegetables are mostly irrigated with animal feces, which may become a potential risk factor for human infection with O157:H7. Therefore, we need to pay attention to the change in this transmission pattern.

In conclusion, national surveillance of EHEC O157:H7 in Xuzhou, where the earliest and largest outbreak in China occurred, has proven to be effective in providing early warning and guidance for assessing the intensity of the disease. Continuous surveillance of the baseline of O157:H7, particularly *stx* virulence trend, is critical for early warning. As a result of these efforts, the monitoring program in Jiangsu Province has been transitioning from O157 to STEC (VTEC) since 2021.

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