Prevalence of *Yersinia enterocolitica* in Pigs Slaughtered in Chinese Abattoirs

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The distribution of *Yersinia enterocolitica* in slaughtered pigs in China was studied. A total of 8,773 samples were collected and examined from different pig abattoirs in 11 provinces from 2009 to 2011. Of these, 4,495 were oral-pharyngeal swab (tonsils) samples from pigs, 1,239 were from intestinal contents, and 3,039 were feces samples from abattoirs or local pigpens. The data showed that 1,132 strains were obtained, from which the isolation rate for *Yersinia enterocolitica* was 19.53% (878/4,495) from the tonsil samples, 7.51% (93/1,239) from intestinal contents, and 5.30% (161/3,039) from feces. Of the 850 pathogenic *Yersinia* strains, except for three of bioserotype 2/O:9 and three of bioserotype 4/O:3, most (844/850) were of bioserotype 3/O:3. Interestingly, pathogenic *Y. enterocolitica* accounted for the majority of the isolated strains from most provinces (85.17% to 100%), whereas from Heilongjiang, 96.52% (111/115) were classified as nonpathogenic biotype 1A with various serotypes, and only 3.48% of the strains (4/115) were pathogenic 3/O:3. All of the pathogenic strains were analyzed using pulsed-field gel electrophoresis (PFGE), and 49 patterns were obtained for the O:3 pathogenic strains; most of them were K6GN11C30021 (53.13%: 450/847) and K6GN11C30012 (21.37%: 181/847). Several strains from diarrhea patient samples revealed PFGE patterns identical to that from samples of local pigs, suggesting a possible link between porcine isolates and human infection. The results above suggested that *Yersinia enterocolitica* in slaughtered pigs from Chinese abattoirs was characterized by region-specific PFGE patterns and confirmed that strains isolated from pigs are closely related to those from human infections.

Yersinia enterocolitica is a zoonotic pathogen widely distributed in nature that can cause acute gastroenteritis and mesenteric lymphadenitis mimicking appendicitis (4). *Y. enterocolitica* is divided into six biotypes (1A, 1B, 2, 3, 4, and 5) and 60 serotypes. Most *Y. enterocolitica* types associated with human yersiniosis belong to bioserotypes 1B/O:8, 2/O:9, 3/O:3, 4/O:3, and 2/O:5,27 (7, 26). *Y. enterocolitica* is recovered from diverse animal sources, from farm animals and domestic pets to free-living and captive wild animals (5, 11). Pigs are considered to be the primary reservoir of human pathogenic *Y. enterocolitica* strains in the world, slaughtered pigs are one of the potentially important sources for the infection (9, 17, 21, 24, 27). A previous study showed that serotypes O:3 and O:9 were the only pathogenic strains isolated from China (30), and it is worth mentioning that the common bioserotype 2/O:9, which followed 3/O:3 in the 1990s, has almost disappeared now (12). In China, the predominant pulsed-field gel electrophoresis (PFGE) patterns for pathogenic *Yersinia* O:3 are K6GN11C30021 and K6GN11C30012; high similarity between strains from slaughtered pigs and patients has been demonstrated by PFGE, indicating that the pigs are the main source of human infection (6, 8). The consumption of pork and products is large in China, with widespread farms breeding domestic pigs and abattoirs processing them. Some abattoirs suffer from bad sanitation conditions and low levels of automatization, where if one swine is infected with *Yersinia enterocolitica*, there is a high risk of transmission of pathogenic *Yersinia* in these enclosed areas. During the slaughter process, the bacteria can be transmitted easily to the carcass and offal and then further to consumers (1, 3, 21). At present, little information is available concerning the distribution

and transmission of *Yersinia enterocolitica* among the slaughtered pigs and patients in China. Thus, we examined several abattoirs located in different regions of China to detect the prevalence of pathogenic *Y. enterocolitica* and studied the geographic distribution, bioserotypes, virulence genes, and prevalent PFGE patterns of pathogenic *Y. enterocolitica* strains.

MATERIALS AND METHODS

Sources of samples. Abattoirs, pigpens, and farms from 11 different provinces of China (Henan [middle]; Sichuan, Yunnan [southwest]; Beijing, the Inner Mongolia Autonomous Region, Tianjin [north]; Heilongjiang [northeast]; the Ningxia Hui Autonomous Region, Qinghai [northwest]; and Jiangsu, Jiangxi [east]) were selected for collection of 8,773 samples from March 2009 to November 2011. Previously published data showed that the isolation rate from tonsil samples was the highest (10). We therefore focused on collecting tonsil samples at the abattoirs (4,495 samples) and intestinal contents at several abattoirs (1,239 samples) and compared them with pig feces samples from pig farms and domestic pigpens (3,039 samples). The largest portion of the tonsil samples was collected in Sichuan and Henan because these areas have the largest population and highest density of pig farms.

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TABLE 1 Primers used in this study

Target gene and primer direction	Primer sequence (5'→3')	Amplicon length (bp)	Annealing temp (°C)
<i>foxA</i>			
Forward	GGT TCC TTG AGC GTA TTG ATG	1,094	58
Reverse	GGT CAT CGG TTT CAG CAG TTT		
<i>ail</i>			
Forward	TAA TGT GTA CGC TGC GAG	351	57
Reverse	GAC GTC TTA CTT GCA CTG		
<i>ystA</i>			
Forward	ATC GAC ACC AAT AAC CGC TGA G	79	61
Reverse	CCA ATC ACT ACT GA CTT CGG CT		
<i>ystB</i>			
Forward	GTA CAT TAG GCC AAG AGA CG	146	61
Reverse	GCA ACA TAC CTC ACA ACA CC		
<i>yadA</i>			
Forward	CTT CAG ATA CTG GTG TCG CTG T	849	60
Reverse	ATG CCT GAC TAG AGC GAT ATC C	759 ^a	
<i>virF</i>			
Forward	GGC AGA ACA GCA GTC AGA CAT A	561	63
Reverse	GGT GAG CAT AGA GAA TAC GTC G		

^a Amplicon length in pathogenic *Y. enterocolitica* serotype O:8.

Culture and PCR. Enrichment was performed using phosphate-buffered saline with sorbitol and bile salts (PSB) at 4°C for 21 days. Nucleic acids from all tonsil samples were extracted using a DNA nucleic acid extraction kit (Tiangen, China). The *Y. enterocolitica* conserved gene *foxA*, the pathogenic gene *ail*, and the *inv* gene of *Yersinia pseudotuberculosis* were amplified (15, 29). The primers and annealing temperatures are shown in Table 1. The tonsil samples positive for either or both of the *foxA* and *ail* genes were then inoculated onto *Yersinia*-selective agar (cef-sulodin-irgasan-novobiocin [CIN] agar; Difco), and all of the intestinal content and fecal samples were also inoculated. The presumptive *Y. enterocolitica* strains with colonies having a typical bull's-eye appearance (deep red centers surrounded by an outer transparent zone) on CIN agar were inoculated onto brain heart infusion (BHI) agar plates incubated at 25°C for 24 h to obtain pure cultures (30, 32).

Bioserotype identification and biochemical tests. The bioserotype identification and biochemical tests of all the strains were performed using the methods reviewed by Wang et al. (31).

Identification of pathogenic *Yersinia enterocolitica* strains. DNA preparation of the strains isolated from the samples and PCR were performed as described previously (31). The relative virulence genes (*ail*, *ystA*, *ystB*, *virF*, and *yadA*) from the chromosome and plasmids were amplified. Pathogenic strains were positive for all (*ail*⁺, *ystA*⁺, *virF*⁺, and *yadA*⁺ strains); however, some pathogenic strains lost the plasmid virulence genes (*ail*⁺, *ystA*⁺, *virF*, and *yadA* strains) (29, 30). The primer sequences and annealing temperatures are shown in Table 1.

PFGE assay. The pulsed-field gel electrophoresis (PFGE) method was used to analyze all of the 850 pathogenic strains isolated in this study, along with 13 samples from diarrhea patients, using the procedures described by Wang et al. (31, 32). The plugs digested with 25 U NotI were electrophoresed with a pulse time from 2 s to 20 s, and then plugs were electrophoresed for 18 to 19 h at 20 V. For data analysis, *.tiff images of the gels were imported into the database of the PFGE patterns of *Y. enterocolitica* strains of China. We performed a cluster analysis for serotypes O:3 and O:9. The patterns of the strains from clinic patients with diarrhea who were from Jiangsu, Sichuan, Tianjin, and Henan Provinces were compared with those pathogenic strains isolated from pigs slaughtered in the same regions. Clustering of the band patterns was performed with Bio-

Numerics software (version 5.1) and with the unweighted-pair group method using average linkages (UPGMA) and Dice coefficient with a 1.5% tolerance. All patterns were visually inspected after computer analysis. The patterns identified as indistinguishable by computer and visual inspections were assigned a pattern designation (31).

RESULTS

Strain isolation. Of the 8,773 samples, 1,132 (12.90%) tested positive for *Y. enterocolitica*, 878 from pig oral-pharyngeal swab (tonsil) samples, 93 from intestinal contents, and 161 from fresh pig feces samples (Fig. 1). The detection rate of *Y. enterocolitica* from tonsil samples by PCR was much higher than cultivation: 36.31% (1,632/4,495) versus 19.53% (878/4,495). In addition, 4 strains of *Yersinia pseudotuberculosis*, 101 strains of *Yersinia frederiksenii/intermedia*, and 16 strains of *Yersinia kristensenii* were isolated in our study (Table 2). The prevalence of *Y. enterocolitica* was shown to be significantly higher from the oral-pharyngeal swabs (19.53%) than from intestinal contents (7.51%) and lowest in pig feces samples (5.30%) (Tables 3 to 5). The prevalence of *Y. enterocolitica* in slaughtered pigs was higher in the north of China than in the southern areas (15.49 versus 6.43%) ($P < 0.01$).

Bioserotype distribution. Among the 850 pathogenic isolates, only three strains of 2/O:9 (3/850) were isolated; O:3 was the primary serotype isolated at 99.65% (847/850), in which serotype all were biotype 3, except for three strains of biotype 4. All of the 282 nonpathogenic strains were biotype 1A (since the serotype identification for nonpathogenic *Y. enterocolitica* was incomplete, we could determine only 93 of the strains; of these, 71 isolates were serotype O:5, and 22 were O:8). The 1,132 *Y. enterocolitica* strains were disproportionately distributed in different areas. Pathogenic *Yersinia* types were primarily isolated from Beijing (100%), Jiangxi (100%), Jiangsu (100%), the Ningxia Hui Autonomous Region (100%), Tianjin (100%), the Inner Mongolia Autonomous Region (93.18%), and Sichuan (89.47%); in contrast, how-

FIG 1 Distribution of *Yersinia* sp. strains isolated from different sources.

ever, from Heilongjiang, located in the northeast of China, only 3.48% (4/115) of pathogenic 3/O:3 strains were isolated, and the rest, 96.52% (111/115), were nonpathogenic biotype 1A (Table 3). We collected 350 pig tonsil and 350 intestinal content samples from the same abattoir (Beijing); the *Y. enterocolitica* isolation rates were 54.57% and 23.14%, respectively, and the proportion of the pathogenic strains in the tonsil samples was 100%, compared to 79.01% (64/81) from the intestinal contents (one strain was detected to be O:9) (Tables 3 and 5).

PFGE. From 847 pathogenic O:3 *Y. enterocolitica* strains, 49 different PFGE patterns were obtained using enzyme NotI, which has been shown to be the most suitable enzyme for characterization of *Y. enterocolitica* in previous studies. The predominant NotI pattern K6GN11C30021 represents 53.13% (450/847) of the strains in pig infections. Patterns K6GN11C30021

and K6GN11C30012 were found in various samples from different provinces, indicating that these patterns are widely distributed in China. More than two types of PFGE patterns were obtained from every abattoir, showing diversities of the strains from Chinese abattoirs. Comparing the patterns before 2009 in our PFGE database, it was found that K6GN11C30012 has existed over the longest period of time, from 1986 to 2011, suggesting that strains with same PFGE pattern could be sustained for many years in the same places and that *Y. enterocolitica* has some genetic stability. Each region has a primary pattern, e.g., K6GN11C30012 in Beijing, Tianjin, and Yunnan and K6GN11C30021 in Henan, Jiangsu, Jiangxi, the Ningxia Hui Autonomous Region, the Inner Mongolia Autonomous Region, Qinghai, and Sichuan. Several regions possess their own specific pattern, such as K6GN11C30044 in Beijing, K6GN11C30042 in Henan, K6GN11C30082 in the

TABLE 2 *Yersinia* sp. strains isolated and samples collected from 11 provinces^a

Province	No. of samples	No. of strains of:			
		<i>Y. enterocolitica</i>	<i>Y. frederiksenii/intermedia</i>	<i>Y. kristensenii</i>	<i>Y. pseudotuberculosis</i>
Beijing	700	272	5	0	0
Tianjin	1,072	67	1	0	0
Heilongjiang	226	115	7	5	0
Henan	1,582	370	29	5	0
Ningxia	1,530	72	1	1	0
Sichuan	827	19	2	0	2
Yunnan	1,215	30	55	0	0
Qinghai	400	26	0	0	0
Jiangxi	102	56	0	0	2
Jiangsu	360	56	0	0	0
Inner Mongolia	759	49	1	5	0
Total	8,773	1,132	101	16	4

^a "0" indicates that no strains were isolated.

TABLE 3 Detection of pig tonsil samples from 11 provinces by both PCR and *Y. enterocolitica* isolation

Province	No. of samples	PCR positive, % ^a	Isolation rate, % ^b	Isolation rate by strain type	
				Pathogenic, % ^c	Nonpathogenic, % ^d
Henan	1,358	31.02	25.33 (344/1,358)	85.17 (293/344)	14.83 (51/344)
Sichuan	827	21.90	2.30 (19/827)	89.47 (17/19)	10.53 (2/19)
Yunnan	667	26.54	3.60 (24/667)	41.67 (10/24)	58.33 (14/24)
Beijing	350	65.14	54.57 (191/350)	100 (191/191)	0
Inner Mongolia	228	36.40	19.30 (44/228)	93.18 (41/44)	6.82 (3/44)
Heilongjiang	226	67.70	50.88 (115/226)	3.48 (4/115)	96.52 (111/115)
Jiangsu	200	38.00	15.00 (30/200)	100 (30/30)	0
Qinghai	200	23.50	11.00 (22/200)	86.36 (19/22)	13.64 (3/22)
Ningxia	192	34.90	13.02 (25/192)	100 (25/25)	0
Tianjin	145	24.14	5.52 (8/145)	100 (8/8)	0
Jiangxi	102	58.82	54.90 (56/102)	100 (56/56)	0
Total	4,495	36.31	19.53 (878/4,495)	79.04 (694/878)	20.96 (184/878)

^a The values are the number positive for either or both *foxA* and *ail*/number of samples.

^b The values in parentheses indicate the number of strains isolated from the samples/number of samples tested.

^c The values in parentheses indicate the number of pathogenic strains/number of strains isolated from the samples.

^d The values in parentheses indicate the number of nonpathogenic strains/number of strains isolated from the samples.

Inner Mongolia Autonomous Region, K6GN11C30058 in Qinghai, and K6GN11C30063 in Sichuan (Fig. 2). Two patterns of the serotype O:9 strains were K6GN11C90003 and one was K6GN11C90004.

The patterns of the strains from patients (three from Jiangsu Province, two from Sichuan Province, and seven diarrhea patients in our database before 2009) were indistinguishable from those of the strains isolated from pigs from the same areas (Fig. 3). K6GN11C30021 was the predominate pattern isolated from pigs and was also found in most infected patients (Fig. 3, area boxed by dotted line).

DISCUSSION

It is well known that *Yersinia enterocolitica* obligately feeds on lymphoid tissue, so it can be easily parasitic on pig throat tonsils, which are rich in lymphoid tissue (14). Pigs (mainly their tonsils) are assumed to be the main reservoirs for pathogenic *Y. enterocolitica* because the pig is so far the only animal species from which pathogenic strains have frequently been isolated (16, 19). In this study, *Yersinia enterocolitica* was isolated from 19.53% (878/4,495) of the tonsil samples, 7.51% (93/1,239) from intestinal contents, and 5.30% (161/3,039) from feces. The isolation rate from

pig tonsil samples was the highest, as observed in European countries (9), indicating tonsils are a more significant source for carcass and offal contamination than feces in slaughterhouses; therefore, tonsil samples were chosen instead of fecal and intestinal content samples in our study to show the distribution of *Y. enterocolitica* in pigs. The detection rate in pig tonsil samples was shown to be even higher using PCR, suggesting plating onto selective medium may underestimate the prevalence of this pathogen in pig (2). Possible causes are fewer strains in the samples and that the *Y. enterocolitica* organisms in samples are already dead as a result of the addition of the antibiotic in the feed: both of these will bring about negative results after cultivation. However, the PCR-based methodology is more sensitive than cultivation, so when detection is with PCR, it may show positivity for *Y. enterocolitica*. In Europe, human yersiniosis is the third most common enteric disease after campylobacteriosis and salmonellosis; however, the positive PCR rate and isolation rate from pigs are no higher than in China (13), yet in China, yersiniosis is sporadic and uncommon. The low number of reported yersiniosis cases in Chinese diarrhea patients could possibly be due to lifestyle and better cooking methods, or perhaps yersiniosis was simply not looked for in such patients. Besides, underreporting in clinics occurs, because our research team de-

TABLE 4 Distribution of *Y. enterocolitica* strains in pig feces samples

Province	Samples	Isolation rate by strain type ^a		
		Pathogenic, %	Nonpathogenic, %	Isolation rate, %
Tianjin	927	100 (59/59)	0	6.36 (59/927)
Henan	224	0	100 (26/26)	11.61 (26/224)
Ningxia	449	14.29 (5/35)	85.71 (30/35)	7.80 (35/449)
Yunnan	548	83.33 (5/6)	16.67 (1/6)	1.10 (6/548)
Qinghai	200	50.00 (2/4)	50.00 (2/4)	2.00 (4/200)
Jiangsu	160	53.85 (14/26)	46.15 (12/26)	16.25 (26/160)
Inner Mongolia	531	20.00 (1/5)	80.00 (4/5)	0.94 (5/531)
Total	3,039	53.42 (86/161)	46.58 (75/161)	5.30 (161/3,039)

^a See Table 3 for explanation of parenthetical data.

TABLE 5 Distribution of *Y. enterocolitica* strains in pig intestinal content samples

Province	Samples	Isolation rate by strain type ^a		
		Pathogenic, %	Nonpathogenic, %	Isolation rate, %
Beijing	350	79.01 (64/81)	20.99 (17/81)	23.14 (81/350)
Ningxia	889	50.00 (6/12)	50.00 (6/12)	1.35 (12/889)
Total	1,239	75.27 (70/93)	24.73 (23/93)	7.51 (93/1,239)

^a See Table 3 for explanation of parenthetical data.

tested an approximately 20% infection rate from infant feces using the PCR method (unpublished data).

The prevalence of *Y. enterocolitica* was significantly different in every province, which shows geographic difference characteristics. Overall, *Y. enterocolitica* incidence in the cold north of China (15.49%: 971/6,269) was much higher than that in the warm southern areas (6.43%: 161/2,504) (Table 2). Some reports also indicate that *Y. enterocolitica* was found more in the cold north region (30, 31). Here we show that the highest isolation rate from tonsil samples (54.9%) was obtained from Jiangxi Province with all of the strains belonging to 3/O:3 (Table 3). This is also the case

FIG 2 Distribution of *Y. enterocolitica* and NotI patterns of 847 serotype O:3 strains recovered from 11 provinces.

FIG 3 Distribution map of pulsed-field gel electrophoresis (PFGE) patterns for pathogenic O:3 serotype isolates from patients and pigs from Jiangsu, Sichuan, Henan, and Tianjin Provinces. Color similarity suggests the patterns of pigs and patients from the same province. The area boxed inside the dotted line shows the predominate pattern K6GN11C30021 isolated from pigs and infected patients.

in the southeastern coast province of Fujian (30), adjacent to Jiangxi Province, with a high incidence. It may be presumed that the distribution of pathogenic *Y. enterocolitica* strains may be related not only to local climate but to other environmental factors as well. This is the first report concerning the prevalence of *Y. pseudotuberculosis* in pigs of China, with an isolation rate of 0.046%

(4/8,773), which was much lower than that from other countries (10, 22). Additionally, among the 8,773 samples, we detected 117 *Yersinia frederiksenii/intermedia* and *kristensenii* samples, most of which were obtained from pig tonsils (Fig. 1). Previous studies showed that the bioserotypes of pathogenic *Y. enterocolitica* in China differ from those in other countries, being

predominantly in 3/O:3 and 2/O:9, with fewer 4/O:3 and no pathogenic 1B/O:8 strains (30, 32). We obtained similar results in this study. In the Ningxia Hui Autonomous Region, 2/O:9 was once the predominant bioserotype in the 1990s (12, 30); however, these strains have gradually decreased in recent years (12). In the United States, 1B/O:8 has also been replaced by O:3 strains, which are now dominant (18, 25). Whether this is occurring due to increasing use of antibiotics in feed or due to immunization needs to be further determined.

The isolation rate shown here from each abattoir varied, and the proportions of pathogenic strains were different. Whereas in Heilongjiang, located in northeast China, despite a high isolation rate of 50.88% (115/226), only 3.48% (4/115) were pathogenic 3/O:3 and 96.52% (111/115) were classified as nonpathogenic biotype 1A (Table 3, Fig. 2). We speculate that different bioserotypes compete and suffer mutual interference in the throats of pigs. The pigs in Heilongjiang were collected from farmers whose own manual feeding is on a small scale, whereas the pigs from Beijing, Tianjin, and other cities were nurtured primarily in big abattoirs, where *Y. enterocolitica* can easily be transmitted among pigs through the fecal-oral route. Whether the different isolation rates are caused by poor feeding models needs to be further studied (20, 22).

The PFGE patterns using NotI enzyme were similar within a given farm, suggesting that specific strains circulate in each herd (Fig. 2). One possible reason is sows in the same areas transmit pathogenic *Yersinia* directly to weaning pigs or indirectly to other pigs when they are in contact with each other via a contaminated environment. The common pattern K6GN11C30021 is found in most human infections and also widely exists in slaughtered pigs (Fig. 3). A previous study revealed that this pattern had existed among animals and diarrhea patients for a long time (31). The PFGE cluster suggested that the patterns of the strains from the patients in the Jiangsu, Sichuan, Tianjin, and Henan Provinces were indistinguishable from those of the strains isolated from pigs from the same areas (Fig. 3). We speculated that the pigs in slaughterhouses may be a source of infection for humans in this region. A possible explanation may be that the *Y. enterocolitica* was directly transmitted from pigs to man through the fecal-oral route or indirectly transmitted from pigs to man via contaminated pork, especially pig offal, and via contaminated environment and pets (31). During a case-control study, a correlation was demonstrated between the consumption of raw or undercooked pork and the prevalence of yersiniosis (23, 28). This shows that the swine is not only the carrier of *Y. enterocolitica* but also the vector causing yersiniosis epidemics and outbreaks. Contamination of pig carcasses during the slaughter process with pathogenic *Yersinia* from tonsils, intestinal contents, and feces may occur. These factors increase the possibility of *Y. enterocolitica* accessing the food chain and multiplying in food products due to its ability to grow at low temperature (30, 31). Meat removed from refrigerators tested positive for *Y. enterocolitica*, and the rate was the highest from pig tongues. Undercooking or cutting raw meat together with edible food may cause human infection.

Domestic dogs belonging to farmers are also a host of *Y. enterocolitica* in China, yet the pathogenic *Y. enterocolitica* is seldom isolated from these or other nonswine animals (31). Previously, many rodents in the Ningxia Hui Autonomous Region of China were detected carrying *Y. enterocolitica*. Most were around farmers' households, so they were in frequent contact with domestic pigs, which may

raise the risk of transmission of pathogenic *Y. enterocolitica* to them (30). Recently, most areas of China have implemented deratting programs, reducing the number of rats significantly, and therefore interinfection with domestic animals (such as swine and dog) rarely happens now. Thus, the isolation rate from rodents has declined, especially during the period from 2009 to 2011; moreover, the mice in the steppe, which is far away from the farmhouses, were negative for *Y. enterocolitica* by culture technology (unpublished data). Therefore, we concluded that swine are the host of *Y. enterocolitica* and the source of infection for humans.

The epidemiological link between pigs and contact with patients is still unclear and has not been documented. This is the first report of the prevalence and dissemination of pathogenic *Y. enterocolitica* in pigs of China. The high prevalence and rapid dissemination of *Y. enterocolitica* in pigs is a challenge for food safety and public health. Further epidemiological investigations are necessary to further determine the reservoir of *Y. enterocolitica* in China and its route of transmission.

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