Low prevalence of Toxoplasma gondii in pork from slaughter houses in the central of China

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Abstract

Background: Toxoplasma gondii is widely distributed and can infect many species of warm-blooded animals , including swine. This study aimed to evaluate the prevalence of T. gondii infection in pork from the cent er of China. A total of 2 798 samples, including 305 hearts, 2086 diaphragm s , and 4 0 7 sera were collected from Henan Province, China . The modified agglutination test was used to detect antibodies against T. gondii in ser a from jugular vein blood and heart blood (cut-off: 1:25) , diaphragm exudate (cut-off: 1:10) . T. gondii DNA was screened from the digestive juice of all diaphragm tissue samples , and attempt to isolate viable T. gondii strain by bioassay in mice.

Results: A total of 9.94% (278/2798) swine showed sera conversion of T. gondii antibodies. Region , but not gender , was associated with T. gondi infection in swine. T. gondii nucleic acid w as not found in the meat digestive juice s (2090 swine) . Three groups of mice showed T. gondii antibodies after having been bioassayed with diaphragm samples (3/81). Unfortunately, no viable T. gondii strain was isolated from pork.

Conclusions : This is the first large-scale survey T. gondi infection in pork from central China. Overall, the prevalence of viable T. gondii in pork was extremely low. Nevertheless, T. gondi i infection is present in swine from center China. Consumers could acquire T. gondii infection from ingestion of raw or undercooked pork.

Background

Toxoplasma gondii is an obligate intracellular parasitic protozoa that is widely distributed worldwide and can infect warm-blooded animals, including swine [1]. Swines are susceptible to *T. gondii* infection and may become infected with *T. gondii* by consumption of oocysts, tachyzoite transplacental transmission, and consumption of meat containing tissue cysts [1]. China is the largest country of producer and consumer of pork, and its annual production was estimated at 55 million metric tons. Pork is known as one of the most common sources of human *T. gondii* infection in China [2].

The overall estimated seroprevalence for *T. gondii* in swine is 32.9% from published papers of 2000–2017 in China, and *T. gondii* strains have been isolated from swine and genotyped as ToxoDB#9, ToxoDB#3, and ToxoDB#1 [3–4]. Overall, the infection of *T. gondii* in swine samples from hospitals is higher than that of farms and slaughterhouses [4]. This phenomenon suggests that *T. gondii* infection may be beneficial for subsequent infection by other pathogens. Few clinical toxoplasmosis in pregnant sows and in fattening swine was reported [4]. Young swine may die from toxoplasmosis without entering the human food chain, however, most *T. gondii* infection in swine was subclinical, and thereby the pork could serve as a source of human infection [1]. However, no pork inspection strategy for *T. gondii* contamination has been established, and no performance standards for processing *T. gondii*-positive meat have been developed. Diaphragm, tongue and heart are the most successful isolation *T. gondii* tissues for the swine [1]. The objective of this investigation was to estimate the seroprevalence of *T. gondii* infection in pork from slaughterhouses, and

try to isolate viable *T. gondii.* To our knowledge, the present study is the first large-scale investigation of *T. gondii* infections in pork from center China.

Results

In the present study, 9.94% (278/2798) (95% CI, 8.88%–11.10%) of the examined swine was seropositive for *T. gondii* infection by MAT (Table 1). A total of 95 sera, four heart, and 179 diaphragm samples were determined to be positive for the *T. gondii* antibody. The seroprevalence of *T. gondii* infection in swine from 13 cities ranged from 0–33%. The seroprevalence rates of *T. gondii* varied with regions. A high prevalence was observed in samples from Xuchang and Xinyang, compared to the other regions ($P < 0.05$), and no seropositive serum from swine was observed in Zhoukou and Puyang.

Risk factors in relation to geographic location and gender were analyzed. The seroprevalence of *T. gondii* by region is shown in Table 2. The seroprevalence of *T. gondii* in swine from the south of Henan (18.23%, 64/391) was higher than that in the center of Henan (7.50%, 103/1,353) and the north of Henan (7.49%, 34/454), with a statistical significance of $P < 0.05$. Only gender information from 235 swine samples was available. Female swine (13.97%, 19/136) showed a tendency to be more susceptible to *T. gondii* (odds ratio = 1.445) than male swine (10.10%, 10/99), but this difference was not significant ($P > 0.05$) (Table 2).

None of 81 groups tissue digestion tissue fluids (4 hearts, 2086 diaphragm) contained *T. gondii* DNA by primers TOX5-TOX8, SAG1 or SAG3 using PCR method (Table 3). For the 81 bioassayed groups, only three groups of mice (Diaphragm group #13, #63, #72) was *T. gondii* positive, detected by MAT at 60 DPI, the titers were above 1:200 for Diaphragm group #13, #63, the titer was 1:25 for Diaphragm group#72 (Table 3). The MAT titer of diaphragm samples from group #63, #72 was all above 1:10. The MAT titer of diaphragm from group #13 was less than 1:10. After sub-passaged, only mice from Diaphragm group #63 showed seroconversion *T. gondii (*MAT >1:200). However, no brain tissue cyst was found at 60 DPI. Unfortunately, viable *T. gondii* was not successfully isolated from pork.

Discussion

T. gondii detection methods include serological methods and etiology assay. Serological methods are more appropriate in detecting *T. gondii* for monitoring meat safety, which could detect specific anti-*T. gondii* immunoglobulin in serum and meat juice within the food chain [5]. However, because the concentration of blood in the meat juice was low compared to that in the serum, it was low sensitivity, and adjusting the dilution factor of meat juice could improve sensitivity [6, 7]. For swine, from serological assays, it has been shown that meat juice from hearts should be diluted to approximately a fifth of the serum dilution; diaphragm samples had *T. gondii* titers less than one tenth of the serum titer [7]. Previous reports have screened for *T. gondii* antibodies in swine sera at a 1:25 dilution or higher by MAT [1, 3], prompting us to use this as a cut-off in the present study. The MAT has been extensively used to detect IgG antibodies to *T. gondii* in sera or body fluids of animals, and its validity was supported by the isolation of *T. gondii* from pigs [1, 3, 8, 9]. We adopted a cut-off value of 1:25 for serum and serum from heart blood, and a cut-off value of 1:10 for the diaphragm exudate. They gave the best agreement with the serum results according

our previous work [8]. However, serological testing has its limitations; for instance, it may fail to detect the active phase of *T. gondii* infection, or the test may not detect *T. gondii* infections in immunocompromised animals. Therefore, testing meat exudate may lead to underestimation of the number of positive samples.

The seroprevalence of *T. gondii* based on screening swine was 11.6% worldwide [1], and 32.9% for China [4]. In this study, 9.94% of the examined swine samples were found to be seropositive for *T. gondii* infection, which is lower than that of the rest of the world as well as China's average infection rate. It is also lower than the seroprevalence of *T. gondii* in free-range chickens, ostriches, sheep, swine, domestic cats, and large cats in Henan Province [10–15]. The reasons for the differences were as follows: the samples in this study were collected from healthy swine in slaughterhouses, improved management and intensive swine farming was developed in China recently. The maximum titer against *T. gondii* antibodies in pork was 1:12800 in this study (one diaphragm exudate), this was higher than that other reports (1:64–1:1024) [1, 16, 17]. After bioassayed analyze in this study, mice from diaphragm with low MAT titer (< 1:10) showed *T. gondii* positive antibody in mice (Table 3). This result indicated that seronegative sample does not guarantee that the pork is free of *T. gondii.*

The seroprevalence of 9.94% in this survey indicates that swine from the central of China are widely exposed to *T. gondii.* The route of *T. gondii* infection in swine is probably by ingestion of *T. gondii* oocysts or cysts. This finding suggests that swine from Henan province had contacted with *T. gondii* oocysts from cats or from soil, water, feed, infected rodents, or cysts from kitchen garbage. The seroprevalence was highest in south of Henan province, suggesting that higher temperature and humid environment favors the survival of oocysts in this region. The results of the present study agree with those of previous reports [18–19]. In this study, female swine were found more susceptible to *T. gondii* than male swine, which may be related to the fact that male swine as breeding pigs are often kept alone, and have less contact with cats and rodents reducing the opportunity of contact with *T. gondii* oocysts. This agreed with prior report in swine [20].

PCR and histopathology were insensitive for the detection of *T. gondii* cysts in pork. PCR could detect trace amounts of *T. gondii* DNA in blood and tissues. However, the prevalence of *T. gondii* chronic infection or natural infection cases is often underestimated due to limited information on the distribution and quantity of parasites. Furthermore, the cysts are not randomly and evenly distributed in tissues [1]. In this study, isolating *T. gondii* DNA from the homogenate of a large meat samples (50 gram or 5 gram meat for one swine) rather than using a small sample would increase PCR sensitivity. However, *T. gondii* DNA was not detected in the pork digestion fluids during this survey (81 tissue homogenate, which came from 2090 swine), which is less than our previously survey (2.06%, 34/1647) from tissue samples in Henan province by *T. gondii* B1 gene [21]. Our result indicated that the relative low density of *T. gondii* parasites in pork.

Bioassays using mice or cats are the gold standard for detecting viable *T. gondii* in meat, which can detect even just a few parasites [1, 22–23]. Bioassays using cats are more sensitive than mice. However, using cats is more expensive and opposed by animal protection organizations. Therefore, the mouse bioassay was used for the present survey. However, bioassays involving mice or cats require at least six weeks of monitoring, which is a relatively long time and thus is not suitable for application to slaughterhouses.

The result of *T. gondii* isolation reports showed that diaphragm, coppa muscle, tongue, and heart are the more viable tissues in swine [16, 24]. The diaphragm and heart were selected in this study, and the sera of three group mice showed *T. gondii* positive antibody. However, no *T. gondii* parasite was observed from mice and no viable *T. gondii* was isolated from pork. However, we could not be sure the safety of pork that only relatively small amounts (50 g or 5 g) of pork have been tested for viable parasites, compared the whole swine (100 Kg). The result may be explained by the relative low density or avirulence of *T. gondii* in pork.

Conclusion

The results of present study showed 9.94% prevalence of *T. gondii* antibody, low *T. gondii* DNA detection rate, and low viable *T. gondii* strainisolated rate was obtained from pork in center China. Considering that pork is the meat with the highest consumption, and its rate of consumption is still steadily growing in China, there is a risk of humans being infected by *T. gondii* through pork. Consumers, human and other carnivores, should take precautions to avoid becoming infected *T. gondii* by eating raw or undercooked pork and swine by-products.

Methods

Investigation site and samples

A total of 2798 fresh swine sampls were collected from 30 slaughterhouses by authors and local veterinarians from March 2015 to November 2017 (Table 1, Figure 1), including 305 hearts (whole), 2086 diaphragms (50 gram), and 407 sera (1 mL) which were separated from jugular vein blood. These swine sampls were from free ranging farms in 13 cities. The swine feed were consisted of commercial feed, kitchen garbage, and fresh vegetables. These swine were males or females, and their ages ranged from 5 to 8 months. The names of the slaughterhouses and sample collection dates were recorded. The samples were allowed us to survey for *T. gondii* infection. Fluide samples (sera, sera from heart blood, and muscle exudate from diaphragm) and tissue samples were stored at 4°C and tested for *T. gondii* antibodies or bioassay in mice within one week.

Assessment of *T. gondii* antibodies in swine samples

The serum, serum from heart blood, and diaphragm exudate samples were tested for antibodies against *T. gondii* by modified agglutination test (MAT) [25]. Serum or serum from heart blood with MAT titers of 1:25 or higher were considered positive for *T. gondii* [1], while diaphragm exudate was 1:10 or higher. Whole formalin-treated *T. gondii* tachyzoite antigens were obtained from the University of Tennessee Research Foundation (Knoxville, TN, USA; https://utrf.tennessee.edu/). *T. gondii*-positive mice sera were provided by Dr. J. P. Dubey (Beltsville, ARS, USA) as reference sera. All samples were tested at 1:10 or 1:25, then the dilution was doubled to the end point titer, positive control and negative control were run on each plate.

Pork processing and bioassaying for viable *T. gondii*

A total of 81 groups of swine tissue samples (myocardium and diaphragm) were bioassayed in mice, and sheep hearts containing *T. gondii* cysts were used as controls (Table 3). The myocardium (50 g) of each of *T. gondii* seropositive swine ((MAT ≥ 1: 25, n = 4, four groups) was bioassayed individually. The diaphragm samples (5 g) of seronegtive swine were pooled (MAT <1:10, 110–120 tissues per group) and bioassayed in mice (n = 1907, 17 groups). The diaphragm (50 g) of seropositive swine were pooled (MAT ≥1: 10, 2–3 tissues per group) and bioassayed in mice (n = 179, 60 groups). Tissue samples were washed, homogenized and digested in pepsin, and the homogenate inoculated subcutaneously into BALB/C mice (n = 5) which drinking water with dexamethasone phosphate (10 μg/ml) 3 days before inoculation [1].

After the inoculation, the clinical symptoms of the mice were observed daily and recorded. Smears of the lungs, mesenteric lymph node, and brain of dead mice were examined for *T. gondii* tachyzoite or cysts within 60 days post inoculation (DPI). After 60 DPI, the mice were bled, and sera were tested for *T. gondii* antibodies using MAT with the dilution of 1:25 and 1:200. Mice were euthanized at 61 DPI, and all of the mice brains were examined for *T. gondii* cysts by squash under a microscope. If tissue cysts were not found in brain smears of seropositive mice, homogenized brain and striated muscle were sub-passaged into new groups of mice subcutaneously. All of the mice were considered to have been successfully infected when *T. gondii* antibodies or parasites were detected in their sera or tissues.

Identification of *T. gondii* DNA by PCR from pork digestive fluids

All of myocardium or diaphragm digestive fluids (four myocardium digestion fluids and 77 diaphragm digestive fluids) were used to detect *T. gondii* DNA. DNA was extracted using a commercial DNA extraction kit (DP304, Tiangen Biotech Company, Beijing, China). PCR assays were performed to detect *T. gondii* using the specific primer pairs TOX5-TOX8, the product from *T. gondii* were expected to be 450 bp in length [26]. Additionally, digested samples were assayed for *T. gondii* by Nested-PCR using primer pairs SAG1 and SAG3, the length of the products was supposed to be 390 bp and 225 bp, respectively, as described previously [27, 28, 29]. The DNA isolated from Me49 *T. gondii* strain (kindly provided by Dr. Dubey) was used as positive control for PCR.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, CA, USA). The data were analyzed using a chi-square test or Fisher's exact test to assess the association between seropositivity and risk factors based on gender (female, male) and geographic location in relation to Henan (south, north, center, east, or west).

Ethics approval and consent to participate

Verbal consent for collecting swine samples from the slaughter houses was obtained from the local veterinarians. The method used in this study is used widely in China, and it was approved by the ethics committee of Henan Agricultural University (China). The protocol was approved by the Beijing Association for Science and Technology (SYXK [Beijing] 2007–0023).

Abbreviations

MAT: modified agglutination test; PCR: polymerase chain reaction.

Declarations

Consent to publish

Not Applicable

Availability of data and material

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests. None of the authors of this report have financial or personal relationships with other people or organizations that could inappropriately influence its content.

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Authors' contributions

RJS performed the laboratory tests. NJ, YYL participated in sample collection and laboratory testing. FCJ, HYW, GPZ, LXZ critically read and revised the manuscript. YRY designed the study protocol, analyzed the results and writing of the manuscript. All authors have read and approved the final version of the manuscript.

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Tables

Table 1 Seroprevalence of *T. gondii* in swine from Henan in China

City	Samples received date	No. of samples	Positive No. in different titers by MAT										% (Positive No./Test No.)
			1:25	1:50	1:100	1: 200	1: 400	1: 800	1: 1600	1: 3200	1: 6400	1: 12800	
Nanyang	09 Mar 2015	30 Sera	3	0	0	7	0	0	0	0	0	0	7.69 (10/130)
	06 May 2015	99 Heart	0	0	0	0	-	-	-	-	-	-	
	13 July 2017	1 Heart	0	0	0	0	-	-	-	-	-	-	
Xinyang	09 Mar 2015	205 Sera	14	4	3	5	7	7	7	4	0	0	24.43 (54/221)
	24 May 2015	1 Heart	1[a]	0	0	0	-	-	-	-	-	-	
	23 June 2015	4 Heart	2[a]	0	0	0	-	-	-	-	-	-	
	01 July 2015	1 Heart	0	0	0	0	-	-	-	-	-	-	
	08 July 2015	2 Heart	0	0	0	0	-	-	-	-	-	-	
	19 Feb 2016	2 Heart	0	0	0	0	-	-	-	-	-	-	
	24 July 2016	6 Heart	0	0	0	0	-	-	-	-	-	-	
Xinxiang	09 Mar 2015	83 Sera	6	2	0	2	0	2	3	3	1	1	24.10 (20/83)
Jiyuan	09 Mar 2015	83 Sera	11	0	0	1	0	0	0	0	0	0	14.46 (12/83)
Puyang	16 April 2015	102 Heart	0	0	0	0	-	-	-	-	-	-	0 (0/102)
Zhengzhou	28 April 2015	3 Heart	0	0	0	0	-	-	-	-	-	-	14.29 (1/7)
	17 Nov 2017	4 Heart	1[a]	0	0	0	-	-	-	-	-	-	
Xuchang	09 Mar 2015	6 Sera	2	0	0	0	-	-	-	-	-	-	33.33 (2/6)
Zhoukou	22 April 2016	80 Heart	0	0	0	0	-	-	-	-	-	-	0 (0/80)

Diaphragm exudate

City	Samples received date	No. of samples	1:10	1:20	1:40	1:80	1:160	1:320	1:640	% (Positive No./Test No.)
Anyang	02 Mar 2017	50	0	0	0	0	0	0	0	1.67 (1/60)
	04 Mar 2017	10	1	0	0	0	0	0	0	
Xinmi	02 Mar 2017	100	1	0	0	0	0	0	0	7.06 (12/170)
	03 Mar 2017	70	10	0	0	0	1	0	0	
Hebi	02 Mar 2017	10	0	0	0	0	0	0	0	5.00 (1/20)
	03 Mar 2017	10	0	1	0	0	0	0	0	
Xinxiang	02 Mar 2017	50	0	0	0	0	0	0	0	6.35 (12/189)
	03 Mar 2017	70	8	2	0	0	0	0	0	
	04 Mar 2017	69	1	0	1	0	0	0	0	
Kaifeng	02 Mar 2017	100	3	0	0	0	0	0	0	14.87 (65/437)
	03 Mar 2017	277	43	9	1	3	1	1	0	
	04 Mar 2017	60	3	1	0	0	0	0	0	
Luohe	02 Mar 2017	20	0	0	0	0	0	0	0	7.14 (10/140)
	03 Mar 2017	70	7	1	0	0	0	0	0	
	04 Mar 2017	50	2	0	0	0	0	0	0	
Pingdingshan	02 Mar 2017	210	12	9	0	0	0	2	0	8.87 (55/620)
	03 Mar 2017	190	3	2	0	0	1	0	1	
	04 Mar 2017	220	15	4	3	3	0	0	0	
Dengfeng	03 Mar	20	3	0	0	0	0	0	0	3.33

	2017									(4/120)
	04 Mar 2017	100	0	0	0	0	0	1	0	
Nanyang	03 Mar 2017	40	0	0	0	0	0	0	0	0 (0/40)
Zhongmou	04 Mar 2017	270	14	3	1	1	0	0	0	7.04 (19/270)
Xinzheng	04 Mar 2017	20	0	0	0	0	0	0	0	0 (0/20)
13 Cities	Summary	2086 Diaphragm	126	32	6	7	3	4	1	8.58 (179/2086)
		305 Hearts								1.31 (4/305)
		407 Sera								23.34 (95/407)
Total										9.94 (278/2798)

[a]: Tissues were bioassay by mice.

"-" It was not tested.

Table 2 Risk factors in region and gender of *T. gondii* infection in swine

Characteristics	No. of tested	No. of seropositive[a]	Prevalence	OR	95% CI	*P* value
Region						
South of Henan	391	64	18.23%			
North of Henan	454	34	7.49%	2.418	1.556-3.756	< 0.0001
Center of Henan	1353	103	7.50%	2.375	1.699-3.320	< 0.0001
East of Henan	517	65	12.57%	1.361	0.9369-1.977	0.1243
West of Henan	83	12	14.46%	1.158	0.5937-2.258	0.7439
Total	2798[b]	278	9.94			
Gender						
Female	136	19	13.97	-	-	-
Male	99	10	10.10	1.445	0.6404-3.262	0.4261
Total	235[c]	29	12.34			

OR: Odds ratio; CI: Confidence interval

[a]: Detection of *T. gondii* antibodies in serum, heart exudate or diaphragm exudate samples by MAT, cut off: 1:25 or 1:10, respectively;

[b]: Total number of samples;

[c]: Total number of samples with gender information.

Table 3 Isolation of *T. gondii* from swine by bioassay in mice

Sample ID	MAT titers	No. positive /No. inoculated	Mice *T. gondii* antibody in different titers by MAT		Passaged groups results[c]
			1:25	1:200	
20150524#1heart	1:25	0/1	-[a]	-	0/1
20150623#1 heart	1:25	0/1	-	-	0/1
20150623#4 heart	1:25	0/1	-	-	0/1
20171117#2 heart	1:25	0/1	-	-	0/1
20170302~04 Diaphragm group #1~12,14~62, 64~71, 73~77	Diaphragm group #1~17 < 1:10; Diaphragm group #18~77 ≥1:10	0/71	-	-	0/1
20170302~04 Diaphragm group #13	All < 1:10	1/1	+/1[b]	+/1[b]	1/3
20170302~04 Diaphragm group #63	1:10; 1:10; 1:160	1/1	+/1[b]	+/1[b]	2/6
20170302~04 Diaphragm group #72	1:10; 1:10; 1:640	1/1	+/1[b]	-	1/3
Total		3/81			

-[a]: Negative;

+/1[b]: one mouse is positive;

[c]: No. of positive groups/No. of passaged groups

Figures

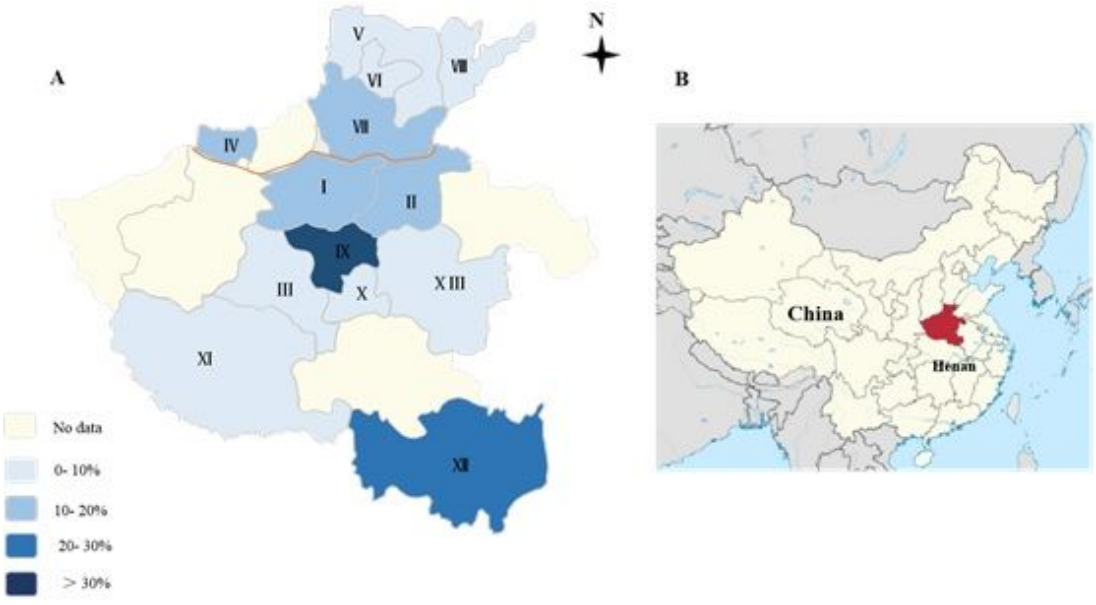

Figure 1

Location of samples received from Henan Province of China. A Cities in Henan province I: Zhengzhou, including Xinzheng, Xinmi, Dengfeng and Zhoumou II: Kaifeng, III: Pingdingshan, IV: Jiyuan, V: Anyang, VI: Hebi, VII: Xinxiang, VIII:Puyang, IX: Xuchang, X: Luohe, XI: Nanyang, XII: Xinyang, XIII: Zhoukou. The yellow line is the Yellow River. B Henan province in China. Figures were adapted from Wikipedia. We would like to thank Wikipedia for providing accurate maps. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.