Low prevalence of antibodies against Toxoplasma gondii in dairy cattle from China’s central region

Hui Dong 1†, Yao Yao Lu 1†, Rui Jing Su 1, Ying Hua Wang 2, Meng Yao Wang 1, Yi Bao Jiang 1 and Yu Rong Yang 1*†

Abstract

Background: Toxoplasma gondii is an intracellular protozoan that can infect humans and other animals, including cattle. Cattle are one of the world’s main sources of meat, and people who consume raw or undercooked meat and milk of cattle infected with T. gondii can become infected. In this study, a total of 5292 dairy cattle serum samples, collected from 17 cities (Henan Province, China) from January 2015 to September 2017, were screened for antibodies against T. gondii.

Results: Antibodies to T. gondii were found in 1.93% (102/5292) (95% CI, 1.56–2.30) of dairy cattle using a modified agglutination test (cut-off 1:100). The results showed that geographic location and season may be risk factors for T. gondii infection of cattle (P<0.05), and the seroprevalence of T. gondii in cattle along the Yellow River is higher than other areas.

Conclusions: This is the first large-scale investigation on the seroprevalence of T. gondii infection in cattle from Central China. This survey shows that the T. gondii infection rate of dairy cattle is low; however, these findings provide additional information on the epidemiology of Chinese T. gondii. The possibility of dairy cattle exposure to T. gondii in Central China cannot be ignored, and the consumption of raw or undercooked beef or milk may pose a risk to human health.

Keywords: Toxoplasma gondii, Cattle, Seroprevalence, Modified agglutination test, Risk factor, Henan Province, China

Background

Toxoplasma gondii is an intracellular protozoan parasite that can infect a wide variety of host species, including cattle [1]. The seroprevalence of T. gondii infection varies among host species. Although cattle appear to be poor hosts for T. gondii, T. gondii can still infect cattle and viable T. gondii strain had been isolated from the intestines of naturally infected cows [2, 3]. Cattle infected with T. gondii pose a risk for toxoplasmosis in people who consume raw or undercooked meat and unpasteurized milk [4]. In humans, T. gondii can cause encephalitis, retinitis, newborn hydrocephalus [1, 5], and even death [6].

Approximately 211 million cattle are raised in China, accounting for 14.4% of the world’s cattle population (National Bureau of Statistics of China, 2015 update).

Surveys regarding T. gondii infection in cattle have been reported in some parts of China. However, only three reports on cattle T. gondii infections in the central region of China have been published to date, which indicated that the prevalence of T. gondii was 20.10% (79/393) in 2011–2013 by IHA (95% CI 16.14–24.06) [7], 5.38% (43/800) in 2011–2012 by IgG test paper (95% CI 3.81–6.94) [8], and 0 (0/102) in 2013–2014 by IHA [9], and there requires more reports as a support to summarize and analyze the epidemiological situation in the region. Large quantities of milk and meat are consumed each year in China, and the safety of the cattle products with respect to T. gondii infection is unknown. The objective of this investigation was to estimate the seroprevalence and risk of T. gondii infection in dairy cattle from Central China. To our knowledge, the present study is the most extensive investigation of T. gondii infections in dairy cattle from Central China.
Results
Our survey indicated that 1.93% (102/5292) (95% CI, 1.56–2.30) of the examined dairy cattle were seropositive for *T. gondii* infection by MAT, with titers of 1:100 in 102 cattle, 1:200 in 40, 1:400 in 21, 1:800 in 17, 1:1600 in 12, and 1:3200 in 7 (Table 1). The seroprevalence of *T. gondii* infection in cattle from 17 cities ranged from 0 to 10%. No information on the geographical locations from 756 cattle samples was available. The seroprevalence rates of *T. gondii* varied with regions. The differences in *T. gondii* seroprevalence among different regions are shown in Table 2. A higher prevalence was observed in the regions of Luoyang and ZhouKou compared to the other regions (*P* < 0.05), and no seropositive sera from cattle were observed in SanMenXia and XinYang.

Risk factors in relation to geographic location and season were analyzed. The prevalence of *T. gondii* in dairy cattle along the Yellow River was higher than in the other areas (Fig. 1). The seroprevalence of *T. gondii* in cattle from south of the Yellow River (3.67%, 57/2116) was higher than that in the north of the Yellow River (1.69%, 41/2420), with a statistically significant odds ratio of 1.606 (95% CI, 1.071–2.410) (*P* = 0.027). In the summer, no positive sera for *T. gondii* were detected in dairy cattle by MAT (*n* = 416). In spring, *T. gondii* antibodies were identified in 3 (0.71%) of 424 samples. In winter, 14 (1.52%) of 921 serum samples tested positive for *T. gondii* antibodies. Out of the 3531 samples collected in autumn, 85 (2.41%) samples were determined to be positive. Seroprevalence was higher in the autumn in contrast to the spring and winter, and the difference between autumn and spring was statistically significant (OR 3.462; *P* = 0.039) (Table 3).

Discussion
It has been estimated that about one third of the world population has been infected with *T. gondii* [5, 11]. Infection is often most common in areas that have hot, humid climates and lower altitudes [1]. Cattle can be readily infected with *T. gondii*, but they are considered poor hosts because these have developed a more effective immune response to *T. gondii* infection than sheep that possibly facilitates in *T. gondii* elimination from tissues, as well as transient antibody responses [12–14]. However, *T. gondii* has been isolated from cattle tissues or unpasteurized milk [13], indicating that meat and milk may be sources of *T. gondii* infections. *T. gondii* has also been detected in cattle semen [1], suggesting it may be transmitted by venereal contact or artificial insemination.

Several test methods have been used in the diagnosis of *T. gondii* infection in humans. However, these could not be applied to cattle based on its incompatibility with the bovine immunoglobulin G system [15]. Current understanding of the specificity and sensitivity of serological diagnosis of *T. gondii* infection in cattle is limited. Furthermore, cattle may not readily acquire persistent *T. gondii* infections, and the actual prevalence rates based on serum antibodies to *T. gondii* are difficult to ascertain. Previous reports have screened for *T. gondii* antibodies in cattle sera at a 1:100 dilution or higher by MAT [1, 16], prompting us to use this as a cut-off in the present study.

Methods

Investigation site and serum samples
Henan Province is located in the central region of China. Henan Province (latitude 34.90°N, longitude 113.50°E) has a humid and subtropical climate. From east to west, the plains transition into the hilly mountains. The average annual temperature is 15.7 °C to 12.1 °C, and the average annual precipitation is 1380.6 to 532.5 mm. The sera of 5292 dairy cattle from 49 farms in 17 cities were collected by local veterinarians from January 2015 to September 2017 (Table 1, Fig. 1). The cattle feed consists of silage, hay, and fresh grass. The cattle were females, and their ages ranged from 2 to 15 years. The farm names and sample collection dates were recorded. The sera were used for Brucellosis screening, which in turn also allowed us to survey for *T. gondii* infection. The cattle sera were separated from jugular vein blood and transported to the Laboratory of Veterinary Pathology, Henan Agricultural University (Zhengzhou, Henan, China) in cooler boxes. The samples were stored at 4 °C and tested for *T. gondii* antibodies within one week.

Assessment for *T. gondii* antibodies
The serum samples were tested for antibodies against *T. gondii* by modified agglutination test (MAT) [10]. Sera with MAT titers of 1:100 or higher were considered positive for *T. gondii* [1]. 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 mouse sera were provided by Dr. J. P. Dubey (Beltsville, ARS, USDA) as reference sera. All serum samples were tested at 1:100, then the dilution was doubled to the maximum titer, and positive controls and negative controls were run on each plate.

Statistical analysis
Statistical analysis was performed using GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA, USA). The data were analyzed using the chi-square test or Fisher’s exact test to assess the association between seropositivity and risk factors based on region (17 cities), season (spring, summer, autumn, and winter), and geographic location (north of the Yellow River, south of the Yellow River).

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| Location/ City | Samples obtained date | Tested No. | No. of seropositive samples /titer | % (Positive No. /Test No.) | 95% CI |
|---------------|-----------------------|-----------|-----------------------------------|-----------------------------|-------|
| I ZhengZhou   | 24 Sep 2015           | 209       | 2                                 | 0.54% (2/369)               | 0.02–2.09 |
|               | 21 Apr 2017           | 30        | –                                 | –                           | –     |
|               | 01 Aug 2017           | 130       | –                                 | –                           | –     |
| II KaiFeng    | 19 Oct 2015           | 42        | 3                                 | 3.49% (3/86)                | 0.77–10.18 |
|               | 24 Sep 2015           | 44        | –                                 | –                           | –     |
| III PingDingShan | 25 Sep 2015         | 300       | 7                                 | 1.98% (7/353)               | 0.88–4.12 |
|               | 27 Jun 2017           | 4         | –                                 | –                           | –     |
|               | 07 Jun 2017           | 49        | –                                 | –                           | –     |
| IV LuoYang    | 22 Sep 2015           | 280       | 31                                | 10.00% (31/310)             | 7.10–13.88 |
|               | 22 Mar 2017           | 30        | –                                 | –                           | –     |
| V AnYang      | 15 Jan 2015           | 369       | 2                                 | 0.41% (2/488)               | 0.01–1.58 |
|               | 05 Jul 2016           | 4         | –                                 | –                           | –     |
|               | 25 Apr 2017           | 32        | –                                 | –                           | –     |
|               | 07 Jun 2017           | 31        | –                                 | –                           | –     |
|               | 28 Jun 2017           | 22        | –                                 | –                           | –     |
|               | 29 Jun 2017           | 30        | –                                 | –                           | –     |
| VI JiaoZuo    | 16 Oct 2015           | 200       | 7                                 | 2.29% (7 /306)              | 1.02–4.74 |
|               | 22 Apr 2017           | 29        | –                                 | –                           | –     |
|               | 26 Jun 2017           | 29        | –                                 | –                           | –     |
|               | 05 Sep 2017           | 48        | –                                 | –                           | –     |
| VII HeBi      | 15 Jan 2015           | 552       | 12                                | 2.07% (12/581)              | 1.14–3.62 |
|               | 15 Jun 2017           | 29        | –                                 | –                           | –     |
| VIII XinXiang | 16 Oct 2015           | 452       | 11                                | 2.28% (11/482)              | 1.23–4.09 |
|               | 22 Mar 2017           | 30        | –                                 | –                           | –     |
| IX PuYang     | 15 Oct 2015           | 250       | 2                                 | 0.71% (2/283)               | 0.02–2.71 |
|               | 05 Sep 2017           | 33        | –                                 | –                           | –     |
| X XuChang     | 25 Sep 2015           | 200       | 5                                 | 1.77% (5/282)               | 0.64–4.20 |
|               | 26 Apr 2017           | 52        | –                                 | –                           | –     |
|               | 27 Apr 2017           | 30        | –                                 | –                           | –     |
| XI LuoHe      | 25 Sep 2015           | 50        | 1                                 | 2.00% (1/50)                | 0.01–11.47 |
| XII SanMenXia | 23 Sep 2015           | 81        | –                                 | –                           | 0 (0/150) – |
|               | 21 Apr 2017           | 23        | –                                 | –                           | –     |
|               | 07 Sep 2017           | 46        | –                                 | –                           | –     |
| XIII NanYang  | 14 Oct 2015           | 170       | 2                                 | 1.06% (3/283)               | 0.21–3.22 |
|               | 21 Mar 2017           | 16        | –                                 | –                           | –     |
|               | 22 Mar 2017           | 39        | –                                 | –                           | –     |
|               | 27 Jun 2017           | 29        | –                                 | –                           | –     |
|               | 07 Sep 2017           | 29        | 1                                 | –                           | –     |
| XIV XinYang   | 18 Apr 2017           | 31        | –                                 | –                           | 0 (0/31) – |
| XV ZhouKou    | 19 Oct 2015           | 50        | 2                                 | 5.63% (4/71)                | 1.80–14.03 |
|               | 21 Mar 2017           | 21        | 2                                 | –                           | 1–1    |
| XVI ZhuMaDian | 25 Apr 2017           | 31        | –                                 | –                           | 0.76% (1/131) 0.01–4.62 |
|               | 29 Jun 2017           | 10        | –                                 | –                           | –     |
Dubey and Jokelainen et al. have conducted *T. gondii* serological testing of cattle [13, 17], and emphasize that the results of *T. gondii* serological screening of cattle should be interpreted with caution. Serology is an indirect method, and the positive results indicate that the host has been exposed to the parasite and produced measurable humoral immune responses. An increase in seroprevalence in cattle up to the age of five years has been reported [17]. The details of the age of the cattle included in the current study were unfortunately unknown, but the age range was wide and did not include only old cattle.

The overall estimated global prevalence of *T. gondii* in cattle using various detection methods is 14.96% (8286/55,377, 1.40–91.80%) [1]. Furthermore, the overall estimated seroprevalence of *T. gondii* in other larger ruminants, specifically water buffalo, horse, and camel was

Table 1 Seroprevalence of *T. gondii* in cattle in Henan Province (Continued)

| Location/ City | Samples obtained date | Tested No. | No. of seropositive samples / (titer) | % (Positive No. / Test No.) | 95% CI |
|---------------|-----------------------|-----------|-------------------------------------|-----------------------------|-------|
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               | 06 Sep 2017           | 90        | 1                                   |                             |       |        |        |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               | XVI JiYuan            | 24 Sep 2015 | 250       | 6                                   | 1     | 1      | 1      | 0.53% (4/756) | 0.15–1.40 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               | XVI NK               | 14 Oct 2015 | 657       | 4                                   | 3     | 1      | 1      | 2.50% (7/280) | 1.11–5.17 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               | XVI NK               | 16 Oct 2015 | 657       | 4                                   | 3     | 1      | 1      | 2.50% (7/280) | 1.11–5.17 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               | XVI NK               | 04 Jul 2017 | 657       | 4                                   | 3     | 1      | 1      | 2.50% (7/280) | 1.11–5.17 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               |                       | 1:100     | 1:200                               | 1:400                       | 1:800 | 1:1600 | 1:3200 |
|               | Total                | 5292      | 102                                 | 40                         | 21    | 17     | 12     | 7     | 1.93% (102/5292) | 1.59–2.34 |

aNK means the city information of samples were not known
bThe samples sampled on same date from a single farm

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**Fig. 1** Location of samples received from Henan Province of China. Figures were adapted from Wikipedia. a Cities in Henan Province I: Zhengzhou, II: Kaifeng, III: Pingdingshan, IV: Luoyang, V: Anyang, VI: Jiaozuo, VII: Hebi, VIII: Xinxiang, IX: Puyang, X: Xuchang, XI: Luoyang, XII: Sanmenxia, XIII: NanYang, XIV: XinYang, XV: Zhoukou, XVI: Zhumaodian, XVII: JiYuan. Yellow line is the Yellow River. b The Yellow River area in China (adapted from Wikipedia). c Henan Province in China (adapted from Wikipedia).
Table 2: P values of comparison of seroprevalence of T. gondii infection in cattle from different cities by Fisher's exact test

| Location       | ZhengZhou | KaiFeng | PingDingShan | LuoYang | AnYang | JiaoZuo | HeBi | XinXiang | PuYang | XuChang | LuoHe | SanMenXia | NanYang | XinYang | ZhouKou | ZhuMaDian |
|----------------|-----------|---------|--------------|---------|--------|---------|------|----------|--------|---------|-------|-----------|---------|---------|---------|-----------|
| KaiFeng        | 0.0489*   | –       | –            | –       | –      | –       | –    | –        | –      | –       | –     | –         | –       | –       | –       | –         |
| PingDingShan   | 0.1003    | 0.4192  | –            | –       | –      | –       | –    | –        | –      | –       | –     | –         | –       | –       | –       | –         |
| LuoYang        | 0.0001*   | 0.0790  | 0.0001*      | –       | –      | –       | –    | –        | –      | –       | –     | –         | –       | –       | –       | –         |
| AnYang         | 1.0000    | 0.0259* | 0.0398*      | 0.0001* | –      | –       | –    | –        | –      | –       | –     | –         | –       | –       | –       | –         |
| JiaoZuo        | 0.0867    | 0.4624  | 0.7940       | 0.0001* | 0.0316* | –       | –    | –        | –      | –       | –     | –         | –       | –       | –       | –         |
| HeBi           | 0.0937    | 0.4263  | 1.0000       | 0.0001* | 0.0271* | 0.8113  | –    | –        | –      | –       | –     | –         | –       | –       | –       | –         |
| XinXiang       | 0.0484*   | 0.4553  | 0.8146       | 0.0001* | 0.0119* | 1.0000  | 0.8350| –        | –      | –       | –     | –         | –       | –       | –       | –         |
| PuYang         | 1.0000    | 0.0851  | 0.3114       | 0.0001* | 0.6271  | 0.1794  | 0.1625| 0.1476   | –      | –       | –     | –         | –       | –       | –       | –         |
| XuChang        | 0.2488    | 0.3963  | 1.0000       | 0.0001* | 0.1067  | 0.7744  | 1.0000| 0.7955   | 0.2856 | –       | –     | –         | –       | –       | –       | –         |
| LuoHe          | 0.3176    | 1.0000  | 1.0000       | 0.1022  | 0.2541  | 1.0000  | 1.0000| 1.0000   | 0.8372 | 1.0000  | –     | –         | –       | –       | –       | –         |
| SanMenXia      | 1.0000    | 0.0473* | 0.1095       | 0.0001* | 1.0000  | 0.1015  | 0.1400| 0.0751   | 0.5461 | 0.1685  | 0.2500| –         | –       | –       | –       | –         |
| NanYang        | 0.6572    | 0.1419  | 0.5244       | 0.0001* | 0.3625  | 0.3433  | 0.4082| 0.2742   | 1.0000 | 0.5043  | 0.4800| 0.5545    | –       | –       | –       | –         |
| XinYang        | 1.0000    | 0.5644  | 1.0000       | 0.0988  | 1.0000  | 1.0000  | 1.0000| 1.0000   | 1.0000 | 1.0000  | –     | 1.0000    | –       | –       | –       | –         |
| ZhouKou        | 0.0073*   | 0.7207  | 0.0942       | 0.3615  | 0.0030* | 0.1332  | 0.0855| 0.1134   | 0.0163*| 0.0845  | 0.2746| 0.0100*   | 0.0322  | 0.3111  | –       | –         |
| ZhuMaDian      | 1.0000    | 0.3032  | 0.6889       | 0.0002* | 0.5106  | 0.4450  | 0.4805| 0.4767   | 0.6695 | 0.4773  | 0.4662| 1.0000    | 1.0000  | 0.01256| –       | –         |
| JiYuan         | 0.0440*   | 0.7051  | 0.7873       | 0.0002* | 0.0139* | 1.0000  | 0.8048| 0.8105   | 0.1050 | 0.5758  | 1.0000| 0.1017    | 0.2203  | 1.0000  | 0.2430  | 0.4450    |

*The difference was considered significant when P value less than 0.05
13.49%, 9.34%, and 35.92%, respectively [1]. The seroprevalence of *T. gondii* based on screening cattle from China is 9.08% (1560/17168, 1.73–46.40%) [18]. In the present study, the seroprevalence of *T. gondii* in cattle was 1.93% (102/5292), which is lower than the rest of the world and China’s average infection rate. It is also lower than the seroprevalence of *T. gondii* in free-range chickens (18.86%, 132/700) [19], ostrich (10.20%, 20/197) [20], sheep (29.33%, 83/283) [21], swine (13.08%, 304/2325) [22], domestic cats (50%, 21/42) [23], and large cats (88.9%, 8/9) [24] in Henan Province. The maximum titer against *T. gondii* antibodies in dairy cattle was 3200 in this study. Considering the effective immune response to *T. gondii* infection in cattle, cattle with high antibody titers may be in the post-acute phase of toxoplasmosis, whereas those with low antibody titers may be in the chronic phase and may contain viable cysts of the parasite in their tissues [1, 25, 26]. However, seronegativity does not guarantee that the meat or milk from cattle will be free of *T. gondii*.

The seroprevalence of 1.93% in this survey indicates that cattle from central China are widely exposed to *T. gondii*. The route of *T. gondii* infection in cattle is probably by ingestion of *T. gondii* oocysts shed by infected felids. This finding suggests that cattle from Henan Province come into contact with *T. gondii* oocysts from cats or from soil, water, or feed. A single *T. gondii* felid could shed millions of oocysts after ingestion of raw meat containing *T. gondii* cysts, and the oocysts can survive in the environment for several years [27]. Furthermore, 100%, 10%, and 71% of cats shed oocysts after primary, secondary, and tertiary infection *T. gondii* [1, 28]. The high seroprevalence of *T. gondii* in domestic cats (50%, 21/42) and wild captured large cats (88.9%, 8/9) from Henan Province [23, 24] suggest that the risk of environmental contamination from felids should be given more attention.

Cattle can acquire *T. gondii* oocysts from the environment by direct contact with soil, feed, or water. The seroprevalence was highest in samples collected in autumn, suggesting that fresh grass used as cattle food may harbor *T. gondii* oocysts, and a humid environment favors the survival of oocysts in this season. The results of the present study agree with those of previous reports [29–31]. However, some studies have shown that season is not a risk factor for *T. gondii* infection [32, 33].

Henan Province is located in the downstream area of the Yellow River. LuoYang and Zhou Kou are much closer to the Yellow River than other cities, and *T. gondii* prevalence in the two regions is relatively higher (*P* < 0.05, Fig. 1). *T. gondii* oocysts can be transported via freshwater runoff into the ocean, thereby posing a threat to humans and other animals residing close to the river [34]. In the present study, risk factor analysis showed that geographic location is associated with *T. gondii* seroprevalence. The seroprevalence of *T. gondii* infection in the cattle from south of the Yellow River was higher than that of the north of the Yellow River (*P* < 0.05), which may be attributable to the mountainous geographic environment and higher temperature.

To our knowledge, neither natural cases of toxoplasmosis nor isolated strains of *T. gondii* in Chinese cattle have been reported to date. Cattle are considered poor hosts for *T. gondii* and good hosts for *Neospora caninum*. *N. caninum* infection in cattle has been found to be widespread in central China [35, 36], and this may cause reproductive losses in the cattle [37]. The dominant *T. gondii* genotype in China is ToxoDB#9 [38]. Several studies have identified *T. gondii* ToxoDB#9 in swine, domestic cats, and sheep in Henan Province [21, 23, 39, 40]. However, only ToxoDB#225 *T. gondii* has been identified in Chinese cattle [40]. Additional investigations on cattle toxoplasmosis are thus warranted.

**Conclusions**

This is the first large-scale investigation on the seroprevalence of *T. gondii* infection in cattle from central China. The rate of *T. gondii* infection in dairy cattle is
relatively low, and this information may be integrated into the Chinese *T. gondii* epidemiology database. Additionally, geographic location appeared as a risk factor for *T. gondii* seropositivity, with prevalence of *T. gondii* in cattle along the Yellow River higher than those of other areas.

**Abbreviations**
MAT: Modified agglutination test; PCR: Polymerase chain reaction

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**Availability of data and materials**
The datasets used and/or analyzed in this study are available from the corresponding author upon request.

**Authors’ contributions**
HD and YYL performed the laboratory tests, data analysis, and wrote the manuscript; RJS, YHW, MYW, and YBJ participated in sample collection and laboratory testing; and YRY designed the study protocol, analyzed the results, and assisted in writing the manuscript. All authors read and approved the final manuscript.

**Ethics approval**
Verbal consent for collecting samples from the farm animals was obtained. This method is widely used in China and 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).

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have 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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