Molecular identification and epidemiological comparison of Cryptosporidium spp. among different pig breeds in Tibet and Henan, China

Shuangjian Zheng†, Dongfang Li†, Chunxiang Zhou1,2, Sumei Zhang1, Yayun Wu1, Yankai Chang1, Yuancai Chen1, Jianying Huang1, Changshen Ning1, Gaiping Zhang1 and Longxian Zhang1*

Abstract

Background: Cryptosporidium spp. are important zoonotic pathogens infecting a wide range of vertebrate hosts, and causing moderate to severe diarrhoea in humans. Cryptosporidium infections are frequently reported in humans and animals worldwide, but little research has been done on local pig breeds such as Tibetan pigs and Yunan Black pigs and imported pig breeds such as Landrace pigs in China. Therefore, a total of 1089 pig faecal samples from four intensive farms in four areas of China, including Tibetan pigs from Gongbujiangda County (n = 180) and Mainling County (n = 434), Tibet, Yunan Black pigs from Sanmenxia, Henan Province (n = 246), and Landrace pigs from Kaifeng, Henan Province (n = 229), and were screened for the presence of Cryptosporidium with microscopy and nested PCR amplification of the small subunit rRNA gene.

Results: The total infection rate of Cryptosporidium in 1089 faecal samples of three different pig breeds was 2.11% (23/1089), and the infection rates of Tibetan pigs, Yunan Black pigs, and Landrace pigs were 0.49% (3/614), 0.41% (1/246), and 8.30% (19/229), respectively. The prevalence of Cryptosporidium infection was significantly higher in weaned piglets (1–2 months) (4.36%, 21/482) than in younger and older age groups (p < 0.01). Sequence analysis of positive samples revealed that there was no mixed infection in our study population, which included 12 cases of C. suis mono-infections (52.17%, 12/23) and 11 cases of C. scrofarum mono-infections (47.83%, 11/23). C. suis was identified in one pre-weaned piglet (< 1 month) and 11 weaned piglets (1–2 months), while C. scrofarum was only detected in 10 weaned piglets (1–2 months) and one finished pig (> 2 months).

Conclusions: This is the first report on the identification of Cryptosporidium spp. in Tibetan pigs, and our findings also elucidate the occurrence and distribution of Cryptosporidium in three different pig breeds in Tibet and Henan, China. More molecular epidemiological studies are required to better clarify the prevalence and public health significance of Cryptosporidium in different pigs.

Keywords: China, Cryptosporidium, Pig, Zoonotic
Background

Cryptosporidium is an important parasitic pathogen, generally causing self-limiting diarrhea in livestock, wild animals, and humans. However, it may cause severe debilitating disease in immunocompromised patients, especially those with acquired immune deficiency syndrome. The pathogen is transmitted via the fecal-oral route in both humans and animals, usually through the ingestion of contaminated water or food.

There is an extensive genetic variation within the genus Cryptosporidium, with 37 valid Cryptosporidium species recognized to date [1–4]. Of these, C. suis, C. scrofarum (formerly Cryptosporidium pig genotype II), C. parvum, C. muris, C. tyzzeri (formerly Cryptosporidium mouse genotype I), and C. andersoni have been identified from pigs [5, 6]. There are also reports that both C. hominis and C. meleagridis can infect pigs [7–9]. Interestingly, cases of C. suis and C. scrofarum infection have been reported in humans in recent years, suggesting that these two pig-adapted Cryptosporidium species are potentially zoonotic [10–12]. C. suis was first identified in a 24-year old HIV patient in Peru in 2002 [13], and was then identified in HIV patients in Peru and China in 2007 and 2013, respectively [14, 15]. C. suis has also been found in patients with digestive system ailments in the United Kingdom and Madagascar [12, 16]. At present, there has only been one reported case of C. scrofarum infection in humans, which occurred in the Czech Republic in 2009 [17].

Landrace pigs are very famous in the world, mainly because of its good reproductive performance and fast growth, but have poor stress resistance [18]. Some indigenous Chinese pig breeds have lower growth rates and lean meat content, compared with conventional western pig breeds, but they are better able to survive in harsh environments. Yunan Black pigs, whose mother is one of some excellent local pig breeds in Henan, were bred by introducing Duroc pigs lineages in a large proportion (62.5%). Its main characteristics are slow growth but introducing Duroc pigs lineages in a large proportion some excellent local pig breeds in Henan, were bred by environments. Yunan Black pigs, whose mother is one of pig breeds, but they are better able to survive in harsh environments. Thus, they have an average elevation of 3000–6000 m. Tibetan pigs are strongly adapted to the natural environment of the plateaus, including tolerance to cold and hypoxia [19]. As a result of economic development, the breeding mode has been transformed from free-grazing to large-scale feed-lots. Despite the large amounts of data on Cryptosporidium spp. in pigs worldwide, there have been very few studies on the occurrence and distribution of Cryptosporidium spp. in pigs in China. At present, there is no information on the prevalence of Cryptosporidium spp. in Tibetan pigs in Tibet, China. Therefore, the aim of this study was to examine the prevalence, identity, and molecular characteristics of Cryptosporidium spp. in three different pig breeds in Tibet and Henan, China, and to estimate their zoonotic potential.

Methods

Sample collection

From March to June 2016, a total of 1089 fresh faecal samples, each of ~ 20 g, were collected from four intensive farms in four areas of China, and each farm was visited only once (Fig. 1; Table 1). The samples were numbered and the sample details were recorded, including sampling time, geographic information and growth stage. During specimen collection, only the inner portion of each faecal sample was collected to ensure no environmental contamination. Each fresh faecal sample was separately collected into sterile gloves before adding 2.5% potassium dichromate and then placed into containers filled with ice packs and immediately transported to the laboratory. Upon arrival, each specimen was first treated by Sheather’s sugar flotation technique, and then examined by microscopy to detect Cryptosporidium oocysts at a bright-field microscope with 100 × and 400 × magnification. All fecal specimens were stored at 4 °C prior to DNA extraction.

DNA extraction and nested polymerase chain reaction amplification

Prior to DNA extraction, fecal samples were washed with distilled water to remove the potassium dichromate. Subsequently, genomic DNA was extracted from approximately 200 mg of semi-purified product using an E.Z.N.A.R Stool DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) as per the manufacturer’s instructions. The extracted DNA was stored in 200-μl volume of Solution Buffer (supplied with the kit) at −20 °C until use.

Cryptosporidium spp. were identified by nested PCR amplification and sequencing of an approximately 840 bp fragment of the small subunit rRNA (SSU rRNA) gene, as described previously [20]. A PCR product of approximately 1325 bp was first amplified with primers F1: 5′-TTCTAGAGCTATAATACATGCG-3′ and R1: 5′-CCCCATTTCCTTCCGAAAACAGGA-3′. The amplification was performed in 25 μl volume with 1 μl of each DNA sample in 2.5 μl 10 × PCR buffer, 2.5 μl deoxyribonucleotide triphosphates (2 mM each), 1.5 μl MgSO4 (25 mM), 0.5 μl each primer (25 μM), 16 μl double distilled water, and 0.5 μl KOD-Plus amplification enzyme (1 units/μl) (ToYoBo Co., Ltd., Osaka, Japan). The first PCR reaction consisted of an initial heating at 94 °C for 5 min, and 35 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 5 min followed by a final extension at 72 °C for 10 min. A secondary PCR product of about 840 bp was then amplified with primers F2: 5′-GGAGGGGTGGTATTTATTAGATAAAG-3′ and R2: 5′-AAGGAGTAA
GGAGGACACCTCCA-3'. The PCR and cycling conditions were identical to the primary PCR. The secondary PCR products were visualized by staining with Golden View following 1% agarose gel electrophoresis.

Sequence analysis
All of the secondary amplification products were sequenced in both directions on an ABI PRISM 3730 XL DNA Analyzer using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). To ensure sequence accuracy, two-directional sequencing was used. To identify Cryptosporidium species, the resulting sequences were subjected to BLAST analysis against sequences in the GenBank database (http://blast.ncbi.nlm.nih.gov), and aligned using Clustal X 2.1 software (http://www.clustal.org/).

Statistical analysis
The Cryptosporidium infection rates were evaluated using Regression Analysis in SPSS 22.0 software for

| Factors          | No. positive/sample size | Prevalence (%) (95% CI) | Cryptosporidium species (no.) |
|------------------|--------------------------|-------------------------|-------------------------------|
| Collection site  |                          |                         |                               |
| Gongbujiangda County, Tibet | 0/180               | 0 (---)                 | /                             |
| Mainling County, Tibet     | 3/434                  | 0.69 (0.147)           | C. scrofarum (3)             |
| Sanmenxia, Henan          | 1/246                  | 0.41 (0.121)           | C. suis (1)                  |
| Kaifeng, Henan            | 19/229                 | 8.30 (4.70–11.90)      | C. suis (11), C. scrofarum (8) |
| Breed                |                          |                         |                               |
| Tibetan pig             | 3/614                  | 0.49 (0.104)           | C. scrofarum (3)             |
| Yunan Black pig         | 1/246                  | 0.41 (0.121)           | C. suis (1)                  |
| Landrace pig            | 19/229                 | 8.30 (4.70–11.90)      | C. suis (11), C. scrofarum (8) |
| Age group              |                          |                         |                               |
| < 1 month               | 1/467                  | 0.21 (0.063)           | C. suis (1)                  |
| 1–2 months              | 21/482                 | 4.36 (2.53–6.19)       | C. suis (11), C. scrofarum (10) |
| > 2 months              | 1/140                  | 0.71 (0.213)           | C. scrofarum (1)             |
| Total                  | 23/1089                | 2.11 (1.26–2.97)       | C. suis (12), C. scrofarum (11) |
Results

Occurrence of Cryptosporidium

Firstly, only five positive samples (5/1089) were detected by microscopy, and then 23 positive samples (23/1089) indentified by PCR (Table 1). The total infection rate of Cryptosporidium spp. in 1089 fecal samples was 2.11% (95% CI 1.26–2.97). The overall Cryptosporidium infection rate of pigs in Tibet was 0.49% (3/614, 95% CI 0–1.04). The prevalence in Mainling County was 0.69% (3/434, 95% CI 0–1.47), while no Cryptosporidium-positive sample was found in Gongbujiangda County. The total infection rate of Cryptosporidium in Henan was 4.21% (20/475, 95% CI 2.40–6.02), and Kaifeng had a higher rate 8.30% (19/229, 95% CI 4.70–11.90) than Sanmenxia 0.41% (1/246, 95% CI 0–1.21) (p < 0.01). Among the three different pig breeds, the infection rates of Cryptosporidium in local pig breeds including Tibetan pigs and Yunan Black pigs were 0.49% (3/614, 95% CI 0–1.04) and 0.41% (1/246, 95% CI 0–1.21), both lower than 8.30% (19/229, 95% CI 4.70–11.90) in imported Landrace pigs (p < 0.01) (Table 1).

The results showed that the prevalence of Cryptosporidium in weaned piglets (1–2 months) was 4.36% (21/482, 95% CI 2.53–6.19), which was significantly higher than the rates observed in pre-weaned piglets (< 1 month; 0.21%, 1/467, 95% CI 0–0.63) (p < 0.01) and finished pigs (> 2 months; 0.71%, 1/140, 95% CI 0–2.13) (p < 0.05) (Table 1). Among the 1–2 months old pigs infected with Cryptosporidium, there were three Tibetan pigs, one Yunan Black pig and 17 Landrace pigs. Interestingly, there was only one Landrace pig infected with Cryptosporidium in each of the other two ages.

Species distribution

Sequence analysis of the SSU rRNA gene fragment revealed the presence of two Cryptosporidium species: C. suis (n = 12) and C. scrofarum (n = 11), and also showed 100% nucleotide identity to a pig-derived sequence (GenBank accession number: GU254174) and pig-derived sequences (GenBank accession numbers: KU668895 and KP704557) respectively. All sequence data has been deposited in the GenBank database under accession numbers MH174659, MH174663, MH178034, and MH178036.

In Henan, two Cryptosporidium species were indentified, including 12 C. suis-positive samples and 8 C. scrofarum-positive samples, while only 3 C. scrofarum-positive samples were found in Tibet. There were many Cryptosporidium -positive samples in imported Landrace pigs, including 11 C. suis-positive samples and 8 C. scrofarum-positive samples, but fewer Cryptosporidium-positive samples in local pig breeds, including 3 C. scrofarum-positive samples in Tibetan pigs and one C. suis-positive sample in Yunan Black pigs. In addition, C. suis was only indentified in pigs aged < 1 month old (n = 1) and 1–2 months old (n = 11), and C. scrofarum was only indentified in pigs aged 1–2 months old (n = 10) and > 2 months old (n = 1).

Discussion

Among the 23 Cryptosporidium-positive samples identified by PCR, only 5 were detected by microscopy, which may be caused by the low concentration of Cryptosporidium oocyst in fecal specimens. In this study, the Cryptosporidium prevalence in Henan was 4.21%, which is lower than that in many reported areas of China, such as Guangdong (8.33%, 6/72), Zhejiang (14.52%, 18/124), Yunnan (23.00%, 46/200), Shanghai (34.44%, 800/2323), Heilongjiang (55.75%, 63/113), and other regions, but only higher than Shaanxi (3.29%, 44/1337) (Table 2) [21–27]. Surprisingly, the infection rate of Cryptosporidium in Tibet is lower than in any reported areas (Table 2). The present study also indicated that the Cryptosporidium infection rates in both local pig breeds were lower than that in imported pig breed. The observed Cryptosporidium prevalence rates of local pig breeds, including 0.49% in Tibetan pigs and 0.41% in Yunan Black pigs, were also apparently lower than that reported in some foreign pig breeds in North America (17.85–55.74%), Europe (5.36–74.67%), and Asia (59.68–71.43%), and in wild boar in the Czech Republic (16.68%) and Spain (16.75%) (Table 3) [6, 28–39]. Many factors, including management system, specimen size and diagnostic technique, may be also responsible for the differences in the prevalence of Cryptosporidium among different pig breeds and different geographic areas.

Cryptosporidium infections are common in pigs and have been found in all age groups worldwide. However, many studies have shown that the highest rates of Cryptosporidium infection occur in weaned piglets (1–2 months), with the infection rates ranging from 20.63 to 83.72% indentified by PCR [6, 23, 26, 27]. In this study, the prevalence of Cryptosporidium in weaned piglets (1–2 months) was at a lower level, but still consistent with the studies of others that weaned piglets (1–2 months) was higher than that in other two age groups. Therefore, although the age distribution of Cryptosporidium infection rates in pigs has not been clearly concluded up to now, we can generally assume that the rate of Cryptosporidium infection in pigs aged 1–2 months is greater than that of other age groups. It is difficult to explain why previous studies have found that conventionally-reared piglets < 1 month of age are reliably infected under the conditions...
Table 2 Distribution of *Cryptosporidium* species/genotypes amongst pigs in China

| Collection site                  | No. positive/sample size | Prevalence (%) by microscopy or others (95% CI) | No. positive/sample size | Prevalence (%) by PCR (95% CI) | *Cryptosporidium* species (no.) | Reference |
|----------------------------------|--------------------------|-----------------------------------------------|--------------------------|--------------------------------|---------------------------------|-----------|
| Zhejiang                         | 18/124                   | 14.52 (8.23–20.80)                             | C. scrofarum (18)        | Zou et al. (2017) [21]          |                                 |           |
| Guangdong                        | 6/72                     | 8.33 (1.79–14.87)                              | C. scrofarum (6)         | Zou et al. (2017) [21]          |                                 |           |
| Yunnan                           | 46/200                   | 23.00 (17.12–28.88)                            | C. scrofarum (46)        | Zou et al. (2017) [21]          |                                 |           |
| Shaanxi                          | 44/1337                  | 3.20 (2.33–4.25)                               | C. suis (42), C. scrofarum (2) | Lin et al. (2015) [22]          |                                 |           |
| Heilongjiang                     | 6/24                     | 37.98 (31.33–44.63)                            | C. suis (14), C. scrofarum (65) | Yin et al. (2013) [24]          |                                 |           |
| Shanghai and Zhejiang, Shaoxing  | 10/70                    | 14.29 (5.88–22.69)                             | C. scrofarum (10)        | Yin et al. (2011) [25]          |                                 |           |
| Shanghai                         | 800/2323                 | 34.44 (32.50–36.37)                            | C. suis (57), C. scrofarum (6) | Chen et al. (2011) [26]         |                                 |           |
| Henan                            | 111/1350                 | 8.22 (6.76–9.69)                               | C. suis (94), C. scrofarum (14) | Wang et al. (2010) [27]         |                                 |           |
| Total                            | 920/4241                 | 21.69 (20.45–22.93)                            | C. suis (229), C. scrofarum (196) C. suis + C. scrofarum (24) |                                 |           |

examined. The age distribution of *Cryptosporidium* infection may also be affected by a number of other factors, including changes in the intestinal environment of the animals caused by diet or age.

Many studies have shown that *C. suis* and *C. scrofarum* appear to be the main pig-adapted species, and some other *Cryptosporidium* species were also identified in pigs, sometimes with mixed infections. Oddly, there was no mixed infection in our study. In this study, *C. suis* and *C. scrofarum* were both found in Henan, which was the same as that in Shaanxi and other regions that have been reported [22–24, 26, 27] (Table 2), while *C. scrofarum* was only identified in Tibet, which was the same as that in Guangdong and other regions that have been reported [21, 25] (Table 2). So far, mixed infections of *C. suis* and *C. scrofarum* were only reported in Heilongjiang and Shanghai, China [23, 26] (Table 2). *C. suis* and *C. scrofarum* were detected in local Yunnan Black pigs and Tibetan pigs in this study, respectively. However, *C. suis* and *C. scrofarum* were fairly common, and mixed infections of *C. suis* and *C. scrofarum* were reported in some foreign breeds in Japan, Austria, The Czech Republic, and Poland [6, 30, 32, 33, 35] (Tables 3). Interestingly, *C. parvum*, *C. muris*, and mouse genotype strains have also been reported in pigs in several countries [32, 34, 36, 37, 39] (Tables 3). In addition, our results also showed that 12 samples from one pre-weaned piglet (<1 month) and 11 weaned piglets (1–2 months) contained *C. suis*, while 11 samples from 10 weaned piglets (1–2 months) and one finished pig (>2 months) contained *C. scrofarum*. These findings are consistent with a previous report showing that the different *Cryptosporidium* species are age-specific in pigs, and piglets are more susceptible to *C. suis* infection while older pigs are more susceptible to *C. scrofarum* [24]. There are many factors leading to differences in the identification of single or mixed infection, with insufficient testing accuracy being a major cause.

A comparative study found that one *Cryptosporidium*-positive sample was typed as *C. muris* on the basis of Sanger sequencing but was identified as a *C. muris* and *C. tyzzer* mixed infection by high-throughput sequencing, which has a superior depth of coverage, especially for mixed infections in clinical samples [40]. Another study found that *C. suis* and *C. scrofarum* in mixed infections were successfully identified using Illumina sequencing technology [41]. However, to improve identification rates of *Cryptosporidium* species or genotypes in mixed-infection samples, more sensitive tools with greater resolution are required.

Conclusions

In conclusion, although the levels of *Cryptosporidium* infection were lower in three different pig breeds in Tibet and Henan, especially in Tibetan pigs and Yunnan Black pigs, the two unidentified *Cryptosporidium* species, including *C. suis* and *C. scrofarum* are both zoonotic. Importantly, this is the first epidemiological investigation of the prevalence and risk factors of *Cryptosporidium* in Tibetan pigs from Tibet. At present, there is no effective drug treatment or vaccine for porcine cryptosporidiosis. Therefore, measures such as strengthening the
Table 3: Distribution of Cryptosporidium species/genotypes amongst pigs worldwide

| Collection site                  | No. positive/sample size | Prevalence (%) by microscopy or others (95% CI) | No. positive/sample size | Prevalence (%) by PCR (95% CI) | Cryptosporidium species (no.) | Reference |
|---------------------------------|--------------------------|-------------------------------------------------|--------------------------|--------------------------------|------------------------------|-----------|
| Japan                           | 112/344                  | 32.56 (27.58–37.53)                              | 37/62                    | 59.68 (47.12–72.24)            | C. suis (13), C. scrofarum (16), C. suis + C. scrofarum (8) | Yui et al. (2014) [6] |
| central Vietnam                 | 28/193                   | 14.51 (9.49–19.52)                               | 10/14                    | 71.43 (44.36–98.50)            | C. suis (8), C. scrofarum (2) | Nguyen et al. (2013) [28] |
| Danish                          | 350/856                  | 40.89 (37.59–44.19)                              | 56/75                    | 74.67 (64.59–84.74)            | C. suis (18), C. scrofarum (38) | Petersen et al. (2015) [29] |
| Austria                         | 2/44                     | 4.55 (0.0–10.95)                                 | 8/44                     | 18.18 (6.32–30.04)             | C. suis (2), C. scrofarum (3), C. suis + C. scrofarum (3) | Němejc et al. (2013) [30] |
| Australia                       |                          |                                                 |                          |                                |                              |           |
| The Czech Republic              | 6/231                    | 2.60 (0.53–4.66)                                 | 39/231                   | 16.88 (12.02–21.75)            | C. suis (13), C. scrofarum (14), C. suis + C. scrofarum (12) | Němejc et al. (2013) [30] |
| The Czech Republic              | 194/1620                 | 11.98 (10.39–13.56)                              | 353/1620                 | 21.79 (19.78–23.80)            | C. suis (142), C. scrofarum (126), C. suis + C. scrofarum (82), C. parvum (1), C. muris (3) | Němejc et al. (2013) [32] |
| The Czech Republic              | 0/193                    | 0                                               | 32/193                   | 16.58 (11.29–21.87)            | C. suis (13), C. scrofarum (7), C. suis + C. scrofarum (12) | Němejc et al. (2012) [33] |
| The Czech Republic              | 87/413                   | 21.07 (17.12–25.01)                              | 69/413                   | 16.71 (13.09–20.32)            | C. suis (45), C. scrofarum (22), C. muris (2) | Kváč et al. (2009) [34] |
| The Czech Republic, South Bohemia| 38/144                   | 26.39 (19.10–33.67)                              | 38/144                   | 26.39 (19.10–33.67)            | C. suis (2), C. scrofarum (21), C. suis + C. scrofarum (15) | Kváč et al. (2009) [35] |
| Poland                          | 3/129                    | 2.33 (0.49–4.96)                                 | 11/129                   | 8.53 (3.64–13.41)              | C. suis (1), C. scrofarum (8), C. suis + C. scrofarum (2) | Němejc et al. (2013) [30] |
| The Slovak Republic             | 0/56                     | 0                                               | 3/56                     | 5.36 (0–11.44)                 | C. suis (2), C. scrofarum (1) | Němejc et al. (2013) [30] |
| Canada, Prince Edward Island    | 163/633                  | 25.75 (22.33–29.17)                              | 113/633                  | 17.85 (14.86–20.84)            | C. suis (41), C. scrofarum (69), C. parvum (2), Mouse genotype (1) | Buduamoako et al. (2012) [36] |
| Canada, Ontario                 | 54/122                   | 44.26 (35.32–53.20)                              | 68/122                   | 55.74 (46.80–64.68)            | C. scrofarum (26), C. parvum (38) | Farzan et al. (2011) [37] |
| Spain, Zaragoza                 | 32/142                   | 22.54 (15.58–29.49)                              | 26/142                   | 18.31 (11.87–24.75)            | C. suis (10), C. scrofarum (16) | Suárezluengas et al. (2007) [38] |
| Spain, Galicia a                 | 35/209                   | 16.75 (11.64–21.85)                              |                           |                                | C. suis (5), C. scrofarum (19), C. parvum (3) | Garcíapresedo et al. (2013) [39] |
| Total                           | 1069/5120                | 20.88 (19.77–21.99)                              | 943/4376                 | 21.55 (20.33–22.77)            | C. suis (328), C. scrofarum (420), C. suis + C. scrofarum (134), C. parvum (44), C. muris (5), Mouse genotype (1) |           |

*The samples from these two studies came from wild boars.*
breeding management of pigs and improving the sanitary and safe disposal of pig feces are needed to avoid the spread of pathogens. In addition, further molecular epidemiological surveys of Cryptosporidium in pigs, humans, and other animals are also needed to better elucidate the mode and risk of transmission.

Abbreviations
PCR: Polymerase chain reaction; SSU rRNA: Small subunit rRNA

Acknowledgments
We thank Tamsin Sheen, PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Funding
This study was supported in part by the National Natural Science Foundation of China (31330079, 31672548), the National Key Research and Development Program of China (2017YFD0501305, 2017YFD0500400, 2016YFD0500707), the China Agriculture Research System (CARS-35), and the Natural Science Foundation of Henan Province (162300410129). The funding body was solely involved in funding and had no role in the design of the study, the collection, analysis, and interpretation of the data, or in writing the manuscript.

Availability of data and materials
The data supporting the findings are included in the manuscript. Representative nucleotide sequences generated in this study were submitted to the GenBank database under the accession numbers MH174659 and MH178034 for C. suis, MH178036 and MH174663 for C. scrofa. Additional data and materials are available upon request from the corresponding author.

Authors’ contributions
LZ conceived and designed the experiments; SZ, DL and CZ performed the experiments; SZ, LZ, YW and Y-C C analyzed the data; S-M Z, CN, and GZ contributed reagents/materials/analysis tools, SZ, Y-C C and JH wrote the paper. All authors critically read and contributed to the manuscript and approved the final version.

Ethics approval
This study was performed in accordance with the Chinese Laboratory Animal Administration Act of 1988. Before the experiments, the research protocol was reviewed and approved by the Research Ethics Committee of Henan Agricultural University. Permission was obtained from all managers of the study pig farms before the fecal samples were collected.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1 College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, People’s Republic of China. 2 College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China.

Received: 11 November 2018 Accepted: 18 March 2019
Published online: 28 March 2019

References
1. Čondlovićová S, Horčíčková M, Sak B, Kvetňoňová D, Hlášková L, Konečný R, Stanko M, McEvoy J, Kvác M, Cryptosporidium apodeni sp. n. and Cryptosporidium dictri sp. n. (Apicomplexa: Cryptosporidiidae) in Apodemus spp. Eur J Protistol. 2018;63:1–12.
2. Ryan U, Fayer R, Xiao L, Cryptosporidium species in humans and animals: current understanding and research needs. Parasitol. 2014;141:1667.
3. Zahedi A, Durmic Z, Gofton AW, Kuehl S, Austen J, Lawson M, Callahan L, Jardine J, Ryan U, Cryptosporidium homari n. sp. (Apicomplexa: Cryptosporidiidae) from the Guinea pig (Cavia porcellus). Vet Parasitol. 2017;245:92.
4. Kvác M, Vinář G, Jelkovičová J, Horčíčková M, Konečný R, Hlášková L, McEvoy J, Sak B, Cryptosporidium occultus, sp. n. (Apicomplexa: Cryptosporidiidae) in rats. Eur J Protistol. 2018;63:96.
5. Kvác M, Kestlánová M, Pinková M, Kvetňoňová D, Kalinová J, Wagnerová P, Kotková M, Vitovec J, Ditrich O, McEvoy J, Stenger B, Sak B, Cryptosporidium scrofa n. sp. (Apicomplexa: Cryptosporidiidae) in domestic pigs (Sus scrofa). Vet Parasitol. 2013;191:218–27.
6. Yui T, Nakajima T, Yamamoto N, Kon M, Abe N, Matsubayashi M, Shibahara T, Age-related detection and molecular characterization of Cryptosporidium suis and Cryptosporidium scrofa in pre- and post-weaned piglets and adult pigs in Japan. Parasitol Res. 2014;113:559–65.
7. Sheoran A, Wiffen A, Widmer G, Singh P, Tzponi S, Infection with Cryptosporidium hominis provides incomplete protection of the host against Cryptosporidium parvum. J Infect Dis. 2011;205:1119–23.
8. Akioshi DE, Dilo J, Pearson C, Chapman S, Turwmine J, Tzponi S, Characterization of Cryptosporidium meleagridis of human origin passed through different host species. Infect Immun. 2003;71:1828.
9. Darabus G, Olariu R, The homologous and interspecies transmission of Cryptosporidium parvum and Cryptosporidium meleagridis. Pol J Vet Sci. 2003;6:235–8.
10. Xiao L, Moore JE, Ukoh U, Gatei W, Lowery CJ, Murphy TM, Dooley JS, Millar BC, Rooney PJ, Rao JR. Prevalence and identity of Cryptosporidium spp. in pig slurry. Appl Environ Microbiol. 2006;72:4461–3.
11. Plutzer J, Karani P, Genotype and subtype analyses of Cryptosporidium isolates from cattle in Hungary. Vet Parasitol. 2007;146:357–62.
12. Rodager JR, Parsons MB, Wright PC, Rasambainarivo F, Roellig D, Xiao L, Gillespie TR, Complex epidemiology and zoonotic potential for Cryptosporidium suis in rural Madagascar. Vet Parasitol. 2014;207:140–3.
13. Xiao L, Bern C, Arowood M, Sulaiman I, Zhou L, Kawai V, Vivar A, Lal AA, Gilman RH, Identification of the Cryptosporidium pig genotype in a human patient. J Infect Dis. 2002;185:1846–8.
14. Cama VA, Ross JM, Crawford S, Kawai V, Chavez-Valdez R, Vargas D, Vivar A, Ticona E, Pedraza-Díaz S, McLauchlin J, Genetic analysis of Cryptosporidium and Cryptosporidium species and subtypes in HIV-infected persons. J Infect Dis. 2007;196:684–91.
15. Wang L, Zhang H, Zhao X, Zhang L, Zhang G, Guo M, Liu L, Feng Y, Xiao L, Zoonotic Cryptosporidium species and Enteroctozon bienesi genotypes in HIV-positive patients on antiretroviral therapy. J Clin Microbiol. 2013;51:1557.
16. Leoni F, Amar C, Nichols G, Pedraza-Díaz S, McLauchlin J, Genetic analysis of Cryptosporidium from 2414 humans with diarrhoea in England between 1985 and 2000. J Med Microbiol. 2006;55:703–7.
17. Kvác M, Kvetňoňová D, Sak B, Ditrich O, Cryptosporidium pig genotype II in immunocompetent man. Emerg Infect Dis. 2005;11582.
18. Zhang W, Yang F, Liu A, Wang R, Zhang L, Shen Y, Cao J, Ling H, Prevalence and genetic characterizations of Cryptosporidium spp. in pre-weaned and post-weaned piglets in Heilongjiang Province, China. PLoS One. 2013;8:e67564.
24. Yin JH, Yuan ZY, Cai HX, Shen YJ, Jiang YY, Zhang J, Wang YJ, Cao JP. Age-related infection with Cryptosporidium species and genotype in pigs in China. Biomed Environ Sci. 2013;26:492–5.
25. Yin J, Shen Y, Yuan Z, Lu W, Xu Y, Cao J. Prevalence of the Cryptosporidium pig genotype II in pigs from the Yangtze River Delta, China. PLoS One. 2011;6:e20738.
26. Chen Z, Mi R, Yu H, Shi Y, Huang Y, Chen Y, Zhou P, Cai Y, Lin J. Prevalence of Cryptosporidium spp. in pigs in Shanghai, China. Vet Parasitol. 2011;183:113–9.
27. Wang R, Qiu S, Jian F, Zhang S, Shen Y, Zhang L, Ning C, Cao J, Qi M, Xiao L. Prevalence and molecular identification of Cryptosporidium spp. in pigs in Henan, China. Parasitol Res. 2010;107:1489.
28. Nguyen ST, Fukuda Y, Tada C, Sato R, Huynh VV, Nguyen DT, Nakai Y. Molecular characterization of Cryptosporidium in pigs in Central Vietnam. Parasitol Res. 2013;112:187.
29. Petersen HH, Wang J, Katakam KK, Mejer H, Thamsborg SM, Dalsgaard A, Olsen A, Enemark HL. Cryptosporidium, and Giardia, in Danish organic pig farms: seasonal and age-related variation in prevalence, infection intensity and species/genotypes. Vet Parasitol. 2015;214:29–39.
30. Němejc K, Sak B, Kvetňová D, Hanzal V, Janiszewski P, Forejtík P, Rajský D, Ravaszová P, McEvoy J, Kvač M. Cryptosporidium suis and Cryptosporidium scrofarum in Eurasian wild boars (Sus scrofa) in Central Europe. Vet Parasitol. 2013;197:504.
31. Johnson J, Buddle R, Reid S, Armson A, Ryan UM. Prevalence of Cryptosporidium genotypes in pre and post-weaned pigs in Australia. Exp Parasitol. 2008;119:418–21.
32. Němejc K, Sak B, Kvetňová D, Kernerová N, Rost M, Cama VA, Kvač M. Occurrence of Cryptosporidium suis and Cryptosporidium scrofarum on commercial swine farms in the Czech Republic and its associations with age and husbandry practices. Parasitol Res. 2013;112:1143–54.
33. Němejc K, Sak B, Kvetňová D, Hanzal V, Jeniková M, Kvač M. The first report on Cryptosporidium suis and Cryptosporidium pig genotype II in Eurasian wild boars (Sus scrofa) in Central Europe. Parasitol Res. 2013;112:1143–54.
34. Kvač M, Hanzlíková D, Kvetňová D. Prevalence and age-related infection of Cryptosporidium suis, C. murs, and Cryptosporidium pig genotype II in pigs on a farm complex in the Czech Republic. Vet Parasitol. 2009;160:319–22.
35. Kvač M, Sak B, Kvetňová D, Kotlíková J, Kvetňová D. Molecular characterization of Cryptosporidium isolates from pigs at slaughterhouses in South Bohemia, Czech Republic. Parasitol Res. 2009;104:425–8.
36. Buduamoako E, Greenwood SJ, Dixon BR, Barkema HW, Hurnik D, Estey C, McClure JT. Occurrence of Giardia and Cryptosporidium in pigs on Prince Edward Island, Canada. Vet Parasitol. 2012;184:18–24.
37. Farzan A, Parrington L, Collin T, Cook A, Pintar K, Pollari F, Friendship R, Farber J, Dixon B. Detection and characterization of Giardia duodenalis and Cryptosporidium spp. on swine farms in Ontario, Canada. Foodborne Pathog Dis. 2011;8:1207.
38. Suárez-Luengas L, Clavel A, Quilez J, Gofli-Cepero MP, Torres E, Sánchez-Acero C, Del Cacho E. Molecular characterization of Cryptosporidium isolates from pigs in Zaragoza (northeastern Spain). Vet Parasitol. 2007;148:231–5.
39. García-Presedo I, Pedraza-Díaz S, González-Varfella M, Mezo M, Gómez-Bautista M, Ortega-Mora LM, Castro-Hermida JA. Presence of Cryptosporidium scrofarum, C. suis and C. parvum subtypes IIaA16G2R1 and IIaA13G1R1 in Eurasian wild boars (Sus scrofa). Vet Parasitol. 2013;196:497–502.
40. Paparini A, Gofton A, Yang R, White N, Bunce M, Ryan UM. Comparison of sanger and next generation sequencing performance for genotyping Cryptosporidium isolates at the 18S rRNA and actin loci. Exp Parasitol. 2015;151:151–2.
41. Kaupke A, Gawor J, Rzetuńka A, Gromadka R. Identification of pig-specific Cryptosporidium species in mixed infections using illumina sequencing technology. Exp Parasitol. 2017;182:22.