Characteristics of Cryptosporidium transmission in pre-weaned dairy cattle in Henan, China

Running title: Cryptosporidium spp. in dairy cattle

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ABSTRACT

To estimate the prevalence and public health significance of cryptosporidiosis in pre-weaned calves in China, 801 fecal samples from eight farms in seven areas in Henan Province were examined for Cryptosporidium oocysts. The overall infection rate of Cryptosporidium was 21.5%, with the farm in Xinxiang having the highest prevalence (40%). No significant difference in infection rates was observed between seasons. Cryptosporidium spp. were characterized by PCR-restriction fragment length polymorphism (RFLP) analysis of the small subunit (SSU) rRNA gene and DNA sequencing of the 60 kDa glycoprotein (gp60) gene. The SSU rRNA-based PCR identified four Cryptosporidium species, including C. parvum (54/172), C. bovis (65/172), C. ryanae (19/172), C. andersoni (12/172), and the occurrence of infections with mixed species (22/172). The earliest detection of C. bovis was in calves of one week of age, showing that the prepatent period was shorter than the previously stated 10-12 days. Infections with C. parvum peaked in summer, whereas C. bovis dominated in autumn and winter. There was no apparent difference in the age of cattle infected with either C. parvum or C. bovis. Sequencing analysis of the gp60 gene showed all 67 C. parvum samples belonged to subtype IIdA19G1. These findings suggested that the transmission of Cryptosporidium spp. in pre-weaned calves in Henan, China appeared to be different from other areas both at genotype and subtype levels. Further molecular epidemiologic studies (including samples from both calves and humans) are needed to elucidate the transmission dynamics and public significance of C. parvum in cattle in China.
INTRODUCTION

Cryptosporidium spp. are important gastrointestinal agents in a wide spectrum of hosts including humans, other mammals, birds, reptiles, amphibians, and fish. There are extensive genetic variations within the genus Cryptosporidium. In addition to over 20 recognized species of Cryptosporidium, more than 60 Cryptosporidium genotypes with no designated species names have been described (9).

Cattle is the common mammalian species in which Cryptosporidium infection was detected, and pre-weaned calves are considered the most important reservoir for zoonotic infection. Thus far, seven Cryptosporidium species and two genotypes have been identified in cattle, including C. parvum, C. bovis, C. andersoni, C. ryanae, C. felis, C. hominis, C. suis, a C. suis-like genotype, and the Cryptosporidium pig genotype II (41). The former four species are mostly responsible for bovine cryptosporidiosis. Studies conducted in numerous industrialized nations suggest that there is an age-associated distribution of the four common Cryptosporidium spp. Thus, C. parvum is mostly found in pre-weaned calves and is a significant cause of diarrhea (41), whereas C. bovis and C. ryanae usually infect weaned calves and yearlings, with C. bovis being more commonly seen than C. ryanae and both not associated with the occurrence of diarrhea (31). In contrast, C. andersoni is commonly seen in adult cattle and has been associated with gastritis, reduced milk yield and poor weight gain (8).

Subtype analysis based on the sequencing of the 60 kDa glycoprotein (gp60) gene has indicated that IIa is the most prevalent subtype family of C. parvum in calves.
worldwide. It has been widely reported in cattle in the United States, Canada, the United Kingdom, Ireland, Sweden, Germany, Belgium, The Netherlands, Italy, Spain, Portugal, Hungary, Serbia and Montenegro, Slovenia, Japan, India, Australia, and New Zealand (7, 17, 25, 33, 34, 44). Among IIa subtypes, IIaA15G2R1 is the most common subtype in calves and has also been commonly identified in human cases in these countries (44). In contrast, other subtype families such as IId and III were uncommon and were only reported in small numbers of cattle in Spain, Portugal, Belgium, The Netherlands, Sweden, Germany, Hungary, Slovenia, and Serbia and Montenegro (33, 44). The only possible exception is Egypt, where a recent small scale study indicated that one IId subtype (IIdA20G1) of *C. parvum* was prevalent on two dairy farms (3).

Dairy industry plays an important role in the agricultural economy of China. In 2007, the total dairy cattle population was 12.3 million (ranking fourth worldwide) and accounted for 5.0% of the total number of dairy cattle in the world (http://kids.fao.org/glipha/). However, only a few studies genetically analyzed small numbers of *Cryptosporidium* isolates. In these studies, *C. andersoni* (n= 29) was identified in post-weaned or adult dairy cattle (21, 47) and *C. bovis* (n= 4) and *C. ryanae* (n= 1) were identified in pre-weaned calves (11). Considering calves are the most important source of zoonotic *Cryptosporidium* infection, the objective of the present study was to identify the species of *Cryptosporidium* present in pre-weaned calves in Henan Province, which has the largest population of dairy cattle in China.

### MATERIALS AND METHODS
Sample collection and examination. Eight hundred and one fresh fecal samples were collected between August 2008 and November 2009 from pre-weaned dairy cattle on eight farms in seven areas in Henan Province, China (Table 1). One of the farms (Zhengzhou A) was visited four times and 369 samples taken in four different seasons were used to assess the seasonal variation in the prevalence. Cryptosporidium oocysts in fecal materials were concentrated using both the formalin-ethyl acetate sedimentation method and the Sheather’s sugar flotation technique. Cryptosporidium oocysts concentrated by the latter method were detected by microscopy under ×400 magnification. Cryptosporidium-positive samples were stored in 2.5% potassium dichromate at 4 °C prior to being used in molecular biologic characterizations.

DNA extraction. Genomic DNA was extracted from Cryptosporidium-positive feces samples using the E.Z.N.A.® Stool DNA Kit (OMEGA Biotek Inc., Norcross, USA) and the manufacturer-recommended procedures.

Cryptosporidium genotyping and subtyping. Cryptosporidium species were determined by nested PCR amplification of a ~830-bp fragment of the small subunit (SSU) rRNA gene (the primary primers “SSU-F2:TTC TAG AGC TAA TAC ATG CG, SSU-R2:CCC ATT TCC TTC GAA ACA GGA”; the second primers “SSU-F3:GGA AGG GTT GTA TTT ATT AGA TAA AG, SSU-R4:CTC ATA AGG TGC TGA AGG AGT A”) and restriction fragment length polymorphism (RFLP) analysis using restriction enzymes SspI and MboII (Fermentas, Shenzhen, China) (11). The diagnosis of C. parvum, C. bovis, C. ryanae, and C. andersoni were confirmed by
DNA sequencing of PCR product from one sample for each species.

All *Cryptosporidium*-positive samples were also analyzed by a nested PCR targeting the gp60 gene (2). The previously established nomenclature system was used in naming *C. parvum* subtype families and subtypes (44).

**DNA sequence analysis.** Sixty-seven PCR products of gp60 gene and one SSU rRNA PCR product each of *C. parvum*, *C. bovis*, *C. ryanae*, and *C. andersoni* were sequenced on an ABI PRISMTM 3730 XL DNA Analyzer (Applied Biosystems, Foster City, USA), using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequence accuracy was confirmed by two-directional sequencing and by sequencing a new PCR product if necessary. The SSU rRNA and gp60 sequences obtained in this study were aligned with reference sequences downloaded from GenBank using the program ClustalX 1.83 (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). Representative nucleotide sequences have been deposited in the GenBank under accession numbers HQ009805 to HQ009809.

**Statistical analysis.** The chi-square test was used to compare *Cryptosporidium* infection rates. Differences were considered significant when \( \rho < 0.05 \).

**RESULTS**

**Prevalence of *Cryptosporidium* spp.** Microscopic analysis of 801 fecal samples showed the presence of *Cryptosporidium* oocysts in 172 samples (21.5%) on all eight farms (Table 1). The highest infection rate (40%) was observed on farm Xinxiang and the lowest infection rate (10.5%) was seen on farm Luoyang (\( \chi^2 = 23.56; \rho < 0.01 \)).
Cryptosporidium oocysts were first observed in animals of 7 days. The Cryptosporidium infection rate in animals of 1, 2, 3, 4, 5, 6, 7, and 8 weeks was 8.0%, 21.4%, 21.5%, 23.7%, 28.4%, 28.6%, 7.1%, and 22.2%, respectively. The differences in infection rates among age groups were not significant ($\chi^2 = 14.68; \rho > 0.05$) (Fig. 1A).

**Distribution of Cryptosporidium species.** All 172 Cryptosporidium-positive samples produced the expected PCR product of the SSU rRNA gene. RFLP analysis of the PCR products revealed the presence of four Cryptosporidium species, including *C. parvum* (54/172) on four farms, *C. bovis* (65/172) on seven farms, *C. ryanae* (19/172) on five farms, and *C. andersoni* (12/172) on three farms; 22 samples from five farms had concurrent infection of mixed species (Table 1). With the exception of the farm Xinmi, all farms had more than one Cryptosporidium species (Table 1).

*Cryptosporidium bovis* was the dominant species on Farm Zhengzhou A ($\chi^2 = 71.95; \rho < 0.01$), whereas *C. parvum* was commonly seen on farms Zhengzhou B ($\chi^2 = 39.62; \rho < 0.01$) and Shangqiu ($\chi^2 = 10.89; \rho < 0.01$) (Table 1). DNA sequencing of the SSU rRNA gene PCR products confirmed the identification of *C. parvum*, *C. bovis*, *C. ryanae*, and *C. andersoni*.

**Age patterns of Cryptosporidium species.** Cryptosporidium bovis was the most commonly identified Cryptosporidium, responsible for 37.8% of all Cryptosporidium infections. It was found in all weekly age groups examined in this study (Fig. 1B). Cryptosporidium parvum was the second most common species, was detected in seven age groups, and accounted for 31.4% of all Cryptosporidium infections (Fig. 1B). No significant difference was observed in the infection rate of *C. parvum* and *C. bovis*.
bovis among the eight age groups (Fig. 1B). The initial detection of C. bovis and C. ryanae was in calves, aged one week and two weeks respectively (Fig. 1B). In contrast, C. andersoni was first detected in animals of 5 weeks in age. The mixed infections were mostly concentrated in calves 4-6 weeks and 8 weeks of age, with C. parvum commonly seen in mixed infections (Fig. 1B).

Seasonal variation in the distribution of Cryptosporidium spp. The highest infection rate (50%) was seen in summer and the lowest (17.3%) in winter ($\chi^2 = 7.17; \rho > 0.05$) (Fig. 2A). Except for spring, the distribution of Cryptosporidium spp. differed among seasons, with C. parvum dominating in summer and C. bovis in autumn ($\chi^2 = 10.89; \rho < 0.01$) and winter ($\chi^2 = 11.41; \rho < 0.01$) (Fig. 2B).

Subtypes of the Cryptosporidium parvum. Sequences of the gp60 gene were successfully obtained from 54 C. parvum-positive samples and 13 samples of mixed infections (Table 1). All of them belonged to the subtype IIdA19G1 (Table 1).

DISCUSSION

The prevalence of cryptosporidiosis in dairy cattle varies among countries in the world. However, a general trend was observed in many studies: the prevalence of Cryptosporidium declined with increases in age (17, 18, 31, 32). In this study, a 21.5% infection rate was seen in pre-weaned dairy calves, which was much higher than the 5.6% (27/485) in post-weaned and adult dairy cattle reported in a recent Chinese study (21).

RFLP and sequence analyses of the SSU rRNA identified four Cryptosporidium species in the 172 positive samples, namely C. parvum, C. bovis, C. ryanae, and C.
Among the species detected, *C. parvum* and *C. bovis* were the two most common species, with *C. bovis* having a higher infection rate (37.8% vs 31.4%) (Fig. 1B). Previously, results of most studies conducted in numerous countries suggested that *C. parvum* was the predominant *Cryptosporidium* species in pre-weaned calves (Table 2). The only exception was a recent study conducted in Sweden, in which 54 of 73 *Cryptosporidium*-positive samples from pre-weaned calves had *C. bovis* (33). In this study, the youngest calf infected with *C. bovis* was one week old, indicating that the prepatent period of *C. bovis* is shorter than the previously stated 10-12 days (10).

Thus, the distribution of *Cryptosporidium* species in pre-weaned dairy calves in Henan, China appears to be different from that seen in most other countries. The reason for the high occurrence of *C. bovis* in this and the Swedish studies is not clear. Feng et al. (2007) suggested that in *C. parvum*-endemic areas, the high infection rate and shedding intensity of *C. parvum* in pre-weaned calves probably had masked the concurrent infection of these animals by *C. bovis* or *C. ryanae* (11).

Sequence analysis of the gp60 gene has been used extensively in characterizing the molecular epidemiology of cryptosporidiosis in calves and humans (45). In recent years, data generated from numerous studies suggested that IIa was the predominant subtype family of *C. parvum* in calves. Within the IIa family, the subtype IIaA15G2R1 was shown to be the most prevalent *C. parvum* subtype in pre-weaned dairy calves in the United States, Canada, Belgium, The Netherlands, Spain, Portugal, Slovenia, Germany, and Japan (Table 2). Several other *C. parvum* IIa subtypes were predominant in other countries (Table 2). In this study, all 67 gp60 PCR positive samples belonged to one single subtype (IIaA19G1), which was detected previously.
in one case in Hungary (28). There was no nucleotide difference between the
IIdA19G1 isolates of two sources. The source of *C. parvum* in calves in Henan is
unclear. Generally, IId is not as common as the major zoonotic subtype family IIa
(44). Thus, only for Hungary (IIdA19G1 and IIdA22G1), Belgium (IIdA22G1),
Portugal (IIdA17G1), Spain (IIdA23G1), Sweden (IIdA20G1e, IIdA23G1, and
IIdA16G1c), Germany (IIdA22G1), Egypt (IIdA20G1), and Serbia and Montenegro
(IIdA18G1b), members of this subtype family were recorded in small numbers of
dairy calves (3, 4, 23, 30, 33, 44). Previously, the IId subtype family of *C. parvum*
was known mostly as a parasite of sheep and goats in southern Europe (29). The only
exception is a recent study in Egypt, in which it was shown that 23/24 of *C.*
*parvum*-infected pre-weaned dairy calves had excreted IIdA20G1 (3). In China, ten *C.*
*parvum* isolates from pet Siberian chipmunks and hamsters in Henan were identified
as IIdA15G1 (22). Despite its rare occurrence in dairy cattle, the IId subtype family is
common in humans in the Middle East (14, 37), and has also been reported in a few
human cases in Portugal, Ireland, the United Kingdom, Belgium, The Netherlands,
and Australia (44). Thus, parasites of the subtype IId may be responsible for zoonotic
transmission of cryptosporidiosis in some areas.

Results of this study suggested that there was no significant seasonal difference in
*Cryptosporidium* infection in pre-weaned calves. However, there appeared to be a
seasonal shift in the dominant *Cryptosporidium* species in pre-weaned calves, with *C.*
*parvum* peaking in summer and *C. bovis* in autumn and winter. This finding is somewhat
different from the recent observation of *C. bovis* dominance in summer and *C. parvum*
dominance in spring and winter in dairy cattle in New York (38). More large studies in
different areas are needed to determine whether these differences are attributable to

difference in animal management.

In conclusion, results of the present study suggest that the transmission of

*Cryptosporidium* spp. in pre-weaned calves in China is probably different from that in

other countries at both the species and subtype levels. Although *C. parvum* is common

in pre-weaned dairy calves, the public health significance of *C. parvum* identified in

this study is still unclear, as no *C. parvum* infection has been seen in humans in China

(27, 42). In other countries the IIa subtype family has been a more important zoonotic

pathogen than the IId family prevalent in this study. Therefore, more molecular

epidemiologic studies in cattle and humans are needed to understand the transmission

dynamics of *Cryptosporidium* spp. in China and the public health significance of *C.

*parvum* in cattle.

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REFERENCES

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TABLE 1. Infection rates of *Cryptosporidium* determined by microscopy on each farm and the distribution of *Cryptosporidium* species, as determined by PCR-RFLP analysis of the SSU rRNA gene, and *C. parvum* subtypes, as identified by sequence analysis of the gp60 gene.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Sample size</th>
<th>Cryptosporidium positive (%)</th>
<th><em>C. parvum</em> (N)</th>
<th><em>C. bovis</em> (N)</th>
<th><em>C. ryanae</em> (N)</th>
<th><em>C. andersoni</em> (N)</th>
<th><em>C. parvum + C. bovis</em> (N)</th>
<th><em>C. parvum + C. ryanae</em> (N)</th>
<th><em>C. parvum + C. andersoni</em> (N)</th>
<th>Subtype (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiaozuo</td>
<td>75</td>
<td>9 (12%)</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>IIdA19G1 (2)</td>
</tr>
<tr>
<td>Luoyang</td>
<td>86</td>
<td>9 (10.5%)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>IIdA19G1 (3)</td>
</tr>
<tr>
<td>Shangqiu</td>
<td>53</td>
<td>9 (17.0%)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>IIdA19G1 (8)</td>
</tr>
<tr>
<td>Xinmi</td>
<td>33</td>
<td>4 (12.1%)</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Xixian</td>
<td>40</td>
<td>16 (40%)</td>
<td>0</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Zhengzhou A</td>
<td>369</td>
<td>88 (23.8%)</td>
<td>26</td>
<td>36</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>8 IIdA19G1 (34)</td>
</tr>
<tr>
<td>Zhengzhou B</td>
<td>126</td>
<td>33 (26.2%)</td>
<td>19</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>IIdA19G1 (20)</td>
</tr>
<tr>
<td>Zhengzhou</td>
<td>19</td>
<td>4 (21.1%)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>801</td>
<td>172 (21.5%)</td>
<td>54 (31.4%)</td>
<td>65 (37.8%)</td>
<td>19 (11.0%)</td>
<td>12 (7.0%)</td>
<td>6 (3.5%)</td>
<td>4 (2.3%)</td>
<td>3 (1.7%)</td>
<td>9 (1.2%)    IIdA19G1 (67)</td>
</tr>
</tbody>
</table>

392 +: mixed infection.

393 N: number of samples positive.
Table 2. Distribution of Cryptosporidium species/genotypes and subtypes in pre-weaned dairy cattle in different countries

+: mixed infection.
N: number of samples positive.

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of isolates</th>
<th>Cryptosporidium species (N)</th>
<th>C. parvum subtype (N)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>161</td>
<td>C. parvum (138), C. bovis (14), C. ryanae (8), C. andersoni (1)</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>USA</td>
<td>23</td>
<td>C. parvum (6), C. bovis (9), C. ryanae (5), C. bovis + C. ryanae (3)</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>USA</td>
<td>175</td>
<td>C. parvum (175)</td>
<td>IIaA15G2R1 (6)</td>
<td>46</td>
</tr>
<tr>
<td>USA</td>
<td>110</td>
<td>C. parvum (107), C. bovis (3)</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>USA</td>
<td>74</td>
<td>44 C. parvum, 25 C. bovis, 5 C. ryanae</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>Canada</td>
<td>44</td>
<td>C. parvum (44)</td>
<td>IIaA15G2R1 (10), IIaA16G2R1 (9), IIaA16G3R1 (8), IIaA16G1R1 (4), IIaA13G2R1 (2), IIaA17G2R1 (2), IIaA18G3R1 (1)</td>
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</tr>
<tr>
<td>Canada</td>
<td>5</td>
<td>C. parvum (5)</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Belgrade, Serbia and Montenegro</td>
<td>62</td>
<td>C. parvum (62)</td>
<td>IIaA16G1R1 (6), IIIA16 (4), IIaA18G1R1 (2), IIaA20G1R1 (2), IIaA18G1 (2), IIaA17 (2)</td>
<td>23</td>
</tr>
<tr>
<td>Belgium</td>
<td>73a</td>
<td>C. parvum (67), C. bovis (6)</td>
<td>IIaA15G2R1 (52), IIaA16G2R1 (2), IIaA14G2R1 (1), IIaA13G2R1 (1)</td>
<td>12</td>
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<tr>
<td>Demark</td>
<td>90</td>
<td>C. parvum (74), 12 C. bovis (12), C. ryanae (3), atypical isolate (1)</td>
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<td>18</td>
</tr>
<tr>
<td>Hungary</td>
<td>22</td>
<td>C. parvum (21), 1 C. ryanae (1)</td>
<td>IIaA16G1R1 (15), IIaA17G1R1 (3), IIaA18G1R1 (1), IIaA18G1 (1), IIaA12G1 (1)</td>
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</tr>
<tr>
<td>Germany</td>
<td>134a</td>
<td>C. parvum (134)</td>
<td>IIaA15G2R1 (43), IIaA14G2R1 (2), IIaA17G2R1 (2), IIaA18G2R1 (2), IIaA21G0R1 (1), IIaA22G0R1 (1), IIaA16G1R1 (1), IIaA22G1 (1)</td>
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</tr>
<tr>
<td>Slovenia</td>
<td>51</td>
<td>C. parvum (45), C. bovis (3), C. ryanae (3)</td>
<td>IIaA15G2R1 (27), IIaA16G1R1 (6), IIaA13R1 (5), IIaA16R1 (3), IIaA16R2 (2), IIIA18R2 (2)</td>
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</tr>
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<td>Estonia</td>
<td>1c</td>
<td>C. parvum (1)</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>160</td>
<td>C. parvum (160)</td>
<td>IIaA15G2R1 (89), IIaA17G1R1 (14), IIaA16G3R1 (6), IIaA13G2R1 (2), IIaA14G2R1 (2), IIaA17G2R1 (2), IIaA18G4R1 (2), IIaA18R1 (2), IIaA19G2R1 (2), IIaA11G2R1 (1), IIaA12G2R1 (1), IIaA16G1R1 (1), IIaA16G2R1 (1), IIaA18G3R1 (1), IIaA19G1R1 (1), IIaA21G3R1 (1), IIIA24 (1)</td>
<td>43</td>
</tr>
<tr>
<td>Spain</td>
<td>149</td>
<td>C. parvum (147), C. bovis (2)</td>
<td>IIaA15G2R1 (106), IIaA16G3R1 (14), IIaA18G3R1 (8), IIaA16G2R1 (4), IIaA17G2R1 (10), IIaA17G2R1 (10), IIaA17G2R1 (7), IIaA19G2R1 (4)</td>
<td>30</td>
</tr>
<tr>
<td>Country</td>
<td>Number</td>
<td>C. parvum</td>
<td>C. bovis</td>
<td>C. ryanae</td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>-----------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Spain</td>
<td>27</td>
<td>27</td>
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401 Cryptosporidium-positive samples were collected from animals younger than 10 weeks of age. 402 The 134 403 samples were positive for oocysts by microscopic analysis. 404 This isolate was from a < 3-month-old calf. 405 The mean age of cattle was 13 days (range 2-125 days) with only 7% of cattle older than 3 weeks.
Fig. 1. Infection rates and distribution of *Cryptosporidium* spp. in pre-weaned dairy calves in Henan, China. (A) Infection rates of *Cryptosporidium* spp. in calves of one to eight weeks of age. (B) Distribution of *C. parvum*, *C. bovis*, *C. ryanae*, *C. andersoni*, and mixed infections by age. N: number of samples examined.

Fig. 2. Seasonal variation in infection rates of *Cryptosporidium* spp. in pre-weaned dairy calves in Henan, China. (A) Infection rates of *Cryptosporidium* spp. in calves in different seasons. (B) Distribution of *C. parvum* and *C. bovis* in calves by season.
Fig. 1.
Fig. 2.