Global prevalence and risk factors of Cryptosporidium infection in Equus: A systematic review and meta-analysis

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Introduction: Cryptosporidiosis is a zoonotic disease caused by Cryptosporidium infection with the main symptom of diarrhea. The present study performed a metaanalysis to determine the global prevalence of Cryptosporidium in Equus animals.

Methods: Data collection was carried out using Chinese National Knowledge Infrastructure (CNKI), VIP Chinese journal database (VIP), WanFang Data, PubMed, and ScienceDirect databases, with 35 articles published before 2021 being included in this systematic analysis. This study analyzed the research data through subgroup analysis and univariate regression analysis to reveal the factors leading to high prevalence. We applied a random effects model (REM) to the metadata.

Results: The total prevalence rate of Cryptosporidium in Equus was estimated to be 7.59% from the selected articles. The prevalence of Cryptosporidium in female Equus was 2.60%. The prevalence of Cryptosporidium in Equus under 1-year-old was 11.06%, which was higher than that of Equus over 1-year-old (2.52%). In the experimental method groups, the positive rate detected by microscopy was the highest (10.52%). The highest Cryptosporidium prevalence was found in scale breeding Equus (7.86%). The horses had the lowest Cryptosporidium prevalence (7.32%) among host groups. C. muris was the most frequently detected genotype in the samples (53.55%). In the groups of geographical factors, the prevalence rate of Cryptosporidium in Equus was higher in regions with low altitude (6.88%), rainy (15.63%), humid (22.69%), and tropical climates (16.46%).

Discussion: The search strategy use of five databases might have caused the omission of some researches. This metaanalysis systematically presented the global prevalence and potential risk factors of Cryptosporidium infection in Equus. The farmers should strengthen the management of young and female Equus animals, improve water filtration systems, reduce stocking densities, and harmless treatment of livestock manure.

KEYWORDS
Cryptosporidium, Equus, meta-analysis, prevalence, zoonotic diseases
**Introduction**

*Cryptosporidium* is a zoonotic coccidian parasite, which mainly parasitizes in the epithelial cells of the small intestine in vertebrates (Xue, 2021). The life cycle of *Cryptosporidium* in hosts comprises asexual and sexual phases, and finally, the oocysts are shed with faeces. The oocyst containing infective sporozoites can survive for months in moist conditions (Chalmers et al., 2019). The *Cryptosporidium*-infected patients usually display signs of diarrhea and abdominal pain, and other clinical features include nausea, vomiting, and low-grade fever. The occasional signs include myalgia, weakness, malaise, headache, and anorexia. The severity, persistence, and eventual outcome of infection generally depend on the characteristics of *Cryptosporidium* and host factors (Bouzid et al., 2013).

*Cryptosporidium* was examined in mucosal tissues of mice by Tyzzer in 1907 (Zhang and Jiang, 2001). Cryptosporidium was the first detection happened to be in immunodeficient Arabian horse foals in 1978 (Snyder et al., 1978). People didn’t pay attention to the disease at first. At the end of the nineteenth century, the events of death of AIDS patients who were infected with *Cryptosporidium* caused a full attention of cryptosporidiosis (Current et al., 1983). Cryptosporidium is worldwide distributed, and the infections of *Cryptosporidium* in organisms have been reported in more than 70 countries (Xue, 2021).

*Equus* animals are mainly distributed in Eurasia and Africa, roughly divided into three species: horse, donkey and zebra. Horses are now mainly used for entertainment or competition, donkeys are roughly divided into three species: horse, donkey and zebra. Horses and donkeys are mainly used for working purposes (Jian, 2012). Because they are maintained in a close association with their owners and veterinary personnel, *Equus* animals are the important reservoirs for transmission of pathogens (such as *Cryptosporidium hominis* and *Toxoplasma gondii*) to humans and other animals (Alvarado-Esquível et al., 2015; Jian et al., 2016). The cases of *Equus* cryptosporidiosis have been reported in many countries around the world, such as China, Italy, and United States. (Veronesi et al., 2010; Qi et al., 2015; Wagnerová et al., 2016).

At present, the systematic evaluation and analysis of *Equus* cryptosporidiosis are still absent. Therefore, a systematic review and meta-analysis were conducted to evaluate the prevalence of *Equus* cryptosporidiosis in the world. The collected information was used to discuss the factors affecting the infection of *Cryptosporidium* in *Equus* (Page et al., 2021).

**Materials and methods**

**Search strategy**

To evaluate the prevalence of *Cryptosporidium* infections in *Equus* around the world, we performed a comprehensive review of literatures both in Chinese and English published from the beginning of the creation to October 1, 2021. The articles were derived from five databases, including CNKI, VIP Chinese Journal Database, Wanfang Data, PubMed, and ScienceDirect. In the three Chinese databases, the advanced search was carried out using “*Equus* (in Chinese)” and “*Cryptosporidium* (in Chinese)” as keywords. In Science Direct, the keywords “*Cryptosporidium*” and “*Equus*” were used for a search. We used MeSH terms “*Cryptosporidium*” and “*Equine*” and their entry terms, such as “*Horse*”, “*Horse, Domestic*”, “*Domestic Horse*”, “*Domestic Horses*”, “*Horses, Domestic*”, “*Equus* “caballus”, and “*Equus przewalskii*” in PubMed. We used boolean operators “AND” to connect MeSH terms and “OR” to connect the entry terms. Finally, the search formula was “(((*Cryptosporidium* OR *Cryptosporidiums*) AND (((((((*Horse* OR *Horse, Domestic*) OR Domestic Horse) OR *Horses, Domestic*) OR *Equus caballus*) OR *Equus przewalskii*) OR *Horse genus*) OR Horse genus)”. Endnote (X9.2 version) was employed to organize the obtained article information. A protocol for the literature review was devised (Figure 1) in accordance with the PRISMA guidelines.

**Inclusion and exclusion criteria**

As part of the eligibility for inclusion, titles that suggested the topic *Cryptosporidium* in *Equus* were selected. The abstracts from the selected reference titles were reviewed by two independent reviewers to determine if the studies met the inclusion criteria and, if so, the entire articles were reviewed in full. The inclusion criteria for the systematic review and meta-analysis were as follows: (1) the object of research was *Equus*; (2) the deceased individuals were positive for *Cryptosporidium* in the research; (3) the research contained clear information, including the number of sick individuals and the population, the number of positive samples, the location of the test, and the location of sampling; (4) the article should contain a full text; (5) the research must be designed for a cross-sectional extension. Articles that do not meet these criteria were excluded. Unpublished reports, comments and copies were also excluded.

**Data extraction**

The extracted data included article title, first author, publication year, detection method, breeding environment, breeding method, genotypes, sampling year, article quality, detailed geographic and climatic factors, total number, number of positives, age and gender of the research object, geographic location (latitude and longitude), altitude, relative humidity, annual average temperature, annual precipitation, climate, and detection method type. The meteorological data of the years involved were from the China Meteorological Data Service Center (CMDC, http://data.cma.cn/) and national centers of
environmental information (https://www.ncei.noaa.gov/maps/monthly/), such as temperature, rainfall, longitude, latitude, humidity and altitude. The database was established by using Microsoft Excel (version 16.32). Two authors independently extracted and recorded data from each selected study. The differences derived from reviewers or uncertainty about the qualifications of the research were further assessed by another author of this paper. The grouping method is based on previous studies (Wei et al., 2021a; Wei et al., 2021b).

Quality assessment

The quality of the included studies was evaluated according to the criteria based on the recommended grading evaluation, formulation, and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) (Xie and Machado, 2021). The scoring criteria were as follows: (1) whether a clear detection method was employed; (2) whether the sampling animal was clear; (3) whether three or more influencing factors were included; (4) the sample size was greater than enough; and (5) whether the sampling year was clear. Therefore, the studies could be scored between 0 and 5 points.

Statistical analyses

The meta package in R software version 4.0.3 (“R core team, R: A language and environment for statistical computing” R core team 2018) was employed to analyze the data in this study (Zeng et al., 2020). Before performing the meta-analysis, we tested five transformation methods to bring the data closer to a Gaussian distribution, namely no transformation (PRAW), logarithmic transformation (PLN), logit transform (PLOGIT), arcsine transform (PAS), and double arcsine transform (PFT) (Table 1). The conversion rate was based on a Shapiro-Wilk normal test. The W-value close to 1 and the P-value greater than 0.05 was close to the Gaussian distribution criterion. The heterogeneity between studies was calculated by Cochran-Q, I² statistics, and χ² test, the P-value < 0.05 and I² = 50% was used to define the degree of heterogeneity with a statistical significance. According to the heterogeneity of the included articles, a random effect model was selected for analysis (Ni et al., 2020). The forest plots were used for a comprehensive analysis. The funnel plot and Egger’s test were used to evaluate publication bias of studies. When there is publication bias in the included articles, the funnel plot is asymmetric, and the distribution is skewed (Egger et al., 1997). The Egger’s test is expected to have a regression intercept of 0 in the absence of bias (Lin and Chu, 2018). The stability of a study was evaluated by the trim and fill analysis and sensitivity analysis (Wang et al., 2021).

To further study the potential sources of heterogeneity, the individual and multivariate model factors were analyzed to determine factors that affected heterogeneity. The factors included sampling surveys (before 2008 vs. others), diagnostic methods (molecular diagnostics vs. other methods), age ≤1year vs. age >1year, genotype (C. parvum vs. others), gender (female vs. male), feeding method (cage-free vs. scale breeding), host (horse vs. others), country (China vs. others), the quality level of publications (5 points vs. others), longitude (<50° vs. others), latitude (<30° vs. others), annual average rainfall (< 500 mm vs. ≥ 500 mm), annual average temperature (< 10°C vs. ≥ 10°C),
annual average humidity (40%-50% vs. others), altitude (<50 m vs. others), and climate (temperate climate vs. others).

Results

Search results

In this study, 1,182 related articles were identified after searching five databases, and 67 articles were selected after the initial screening and removal of duplicates. According to the selection criteria described in section, an additional 32 indeterminate articles were excluded after checking the full text. Finally, 35 articles were selected for the meta-analysis. (Figure 1; Table 2).

Qualification research and publication bias

The included articles involved 13 countries. In the 35 studies, the total number of samples was 9,817, and the number of positives was 816 (Table 3). According to our quality criteria, 11 articles were considered to be 5 points, 21 were of medium-quality (three or four points), and the remaining 3 articles were deemed to be of low-quality (zero to two points; Table 3).

In the included studies, the forest plot showed the degree of heterogeneity of all data ($\chi^2 = 0.0267, I^2 = 96.0\%, P < 0.01$) (Figure 2). According to the funnel chart, the distribution of points was observed to be incompletely symmetrical, which might be due to a publication bias (0.6344) or a small sample bias (Figure 3). Six supplementary studies were found in trim and fill analysis, which changed the aggregate estimate (Figure 4). The Egger’s test was used to assess the potential publication bias in the analysis with a $P$-value (0.7398) greater than 0.05, thus indicating that no publication bias was present in the data (Figure 5). The sensitivity test showed that the reorganized data were not significantly affected after excluding any study, and the results were consistent (Figure 6), which verified the rationality and reliability of this analysis. Figure 7 is a map of Cryptosporidium prevalence in Equus worldwide. A chord diagram shows the relationship between the prevalence of Cryptosporidium in Equus species and epidemiological variables (stripe width indicates prevalence) (Figure 8). C. parvum genotype had a larger proportion in North America in the geographic area grouping. Genotype C. hominis accounted for a large proportion of donkey in the host group. Genotype C. andersoni accounted for more than 1-year-old in the age group.

Meta-analysis of Equus Cryptosporidium worldwide

According to the data obtained from the selected articles, the global combined prevalence of Equus Cryptosporidium infection was 7.59% (95% CI: 4.86-10.87) (Table 3). Before 2008, the prevalence of Cryptosporidium in Equus was 10.77% (95% CI: 3.92-20.32), which was significantly higher than that in other time periods ($P < 0.01$). The highest positive rate of Equus Cryptosporidium was 17.91% (95% CI: 9.73-27.92) in Oceania. Among the detection methods, microscopic examination had the highest rate of 10.52% (95% CI: 4.99-17.19). The prevalence of Cryptosporidium in Equus ≤ 1-year-old was 11.06% (95% CI: 6.13-17.22), which was significantly higher than in other age groups ($P < 0.01$). The highest prevalence of Cryptosporidium in female Equus was 2.60% (95% CI: 0.78-5.44). The highest prevalence of Cryptosporidium among the rearing method groups was 7.86% (95% CI: 4.39-12.22) for collectivized breeding. Among the host groups, mules had the highest rate of 20.00% (95% CI: 10.20-32.09). The highest prevalence of Equus Cryptosporidium was 19.58% (95% CI: 6.03–38.43) in Italy, which was significantly higher than that in other countries (Table 4). The highest genotype prevalence of Cryptosporidium in Equus was C. muris (53.55%; 95% CI: 11.65-92.51), followed by C. hominis, with a rate of 43.94% (95% CI: 23.11–65.95) (Table 5).

Among the analyzed geographical factors, the positive rate of Cryptosporidium in Equus at northern latitude < 30° was the highest (8.77%; 95% CI: 2.88-17.44). The positive rate of Cryptosporidium in Equus with longitude > 100° was the highest (9.03%, 95% CI: 3.15-17.53). The information for other geographical latitude subgroup analyses included precipitation range (≥ 500 mm; 15.63%; 95% CI: 8.60-24.28), temperature range (≥ 10°C; 8.04%, 95% CI: 3.30-14.60), humidity range

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**TABLE 1** Normal distribution tests for normal rates and different transitions.

| Conversion form | W   | P         |
|----------------|-----|-----------|
| PRAW           | 0.8277 | 7.482e-05 |
| PLN            | NaN  | NA        |
| PLOGIT         | NaN  | NA        |
| PAS            | 0.94712 | 0.09241   |
| PFT            | 0.94693 | 0.09118   |

"PRAW": raw exchange rate; PLN: log conversion; "PLOGIT": logit transformation; "PAS": arcsine transformation; "PFT": double arcsine transformation; "NaN": meaningless number; NA: data is missing.
Discussion

Cryptosporidium is a waterborne pathogen that infects livestock, poultry, and companion animals, thus posing a great threat to public health (Gao et al., 2021). Many large Cryptosporidium outbreaks were caused by contamination of water sources with animal feces (Rodriguez et al., 2012). Cryptosporidiosis can cause slow growth of sick animals, extreme weight loss, decreased resistance, and huge losses to the animal husbandry (Han et al., 2020). In this study, a publication bias was observed according to the Egger’s test. Pass I² statistics results, the prevalence of Cryptosporidium in Equus species in the world was highly heterogeneous in the eligible studies, which may be caused by differences in detection methods, age, gender, geographic factors, and countries.

The prevalence of Cryptosporidium in female Equus was identified to be higher than that in male Equus. This is

| Study ID          | Sampling time | Country     | Test method                        | Event | Positive rate |
|-------------------|---------------|-------------|------------------------------------|-------|---------------|
| Xiao L et al. (1994) | 1992.3-1992.10 | America     | Direct immunoﬂuorescence staining method | 16/222 | 0.072         |
| Johnson et al. (1997) | 1994.8-1994.10 | America     | Direct fluorescent antibody         | 0/91  | 0.000         |
| Olsson et al. (1997) | 1996.7        | Canada      | Microscopic examination             | 6/35  | 0.171         |
| Majewska et al. (1999) | NA           | Poland      | Enzyme immunoassay                  | 10/106| 0.094         |
| Majewska et al. (2004) | NA           | Poland      | Microscopic examination             | 11/318| 0.035         |
| Grinberg et al. (2009) | 2005-2007     | New Zealand | Microscopic examination             | 12/67 | 0.179         |
| De Souza et al. (2009) | NA           | Brazil      | Microscopic examination             | 3/396 | 0.008         |
| Verronesi et al. (2010) | 2007.2-4      | Italy       | DFA                                 | 12/150| 0.080         |
| Burton et al. (2010) | 2009.2-5      | America     | DFA                                 | 16/349| 0.046         |
| Ferrucci et al. (2011) | 2006-2008    | NA          | ELISA                              | 2/74  | 0.027         |
| Jian (2012)         | 2008.7-2013.9 | China       | Microscopic examination             | 222/1302 | 0.171 |
| Inácio et al. (2012) | 2010.11-2011.3 | NA        | Microscopic examination             | 39/196 | 0.199         |
| Caffara et al. (2013) | NA           | Italy       | PCR                                | 14/37 | 0.378         |
| Laatamna et al. (2013) | 2010-2011    | Algeria     | PCR                                | 4/138 | 0.029         |
| Guo P et al. (2014)  | 2001.9-2003.10 | China       | PCR                                | 161/436 | 0.369 |
| Qi et al. (2015)     | 2013.8-9      | China       | PCR                                | 7/262 | 0.027         |
| Liu A et al. (2015)  | NA            | China       | PCR                                | 3/29  | 0.103         |
| Laatamna et al. (2015) | 2011.11-2013.5 | Algeria    | PCR                                | 7/343 | 0.020         |
| Kostopoulou et al. (2015) | NA         | Belgium et al. | PCR                            | 8/398 | 0.020         |
| Zhou H. (2015)      | 2013.3-2014.5 | China       | Microscopic examination             | 30/508 | 0.059         |
| Galoppo et al. (2015) | 2011.12-2012.12 | Italy     | PCR                                | 14/73 | 0.192         |
| Wagnerová et al. (2015) | 2011-2012    | NA          | PCR                                | 12/352 | 0.034        |
| Wagnerová et al. (2016) | NA           | America     | PCR                                | 28/84 | 0.333         |
| Hijawí et al. (2016) | 2014.10-2015.5 | NA        | PCR                                | 6/74  | 0.081         |
| Song Y et al. (2017) | NA            | China       | PCR                                | 1/10  | 0.100         |
| Deng L et al. (2017) | 2015.8-2016.4 | NA          | PCR                                | 6/333 | 0.018         |
| Inácio et al. (2017) | 2010.11-2011.3 | Brazil     | PCR                                | 20/92 | 0.217         |
| Raue et al. (2017)   | 2004-2012     | Germany     | Microscopic examination             | 4/21  | 0.190         |
| Deng (2018)         | 2015.7-2017.5 | China       | PCR                                | 6/441 | 0.014         |
| Zhang Q et al. (2019) | 2018.5-7     | China       | PCR                                | 0/32  | 0.000         |
| Li F et al. (2019)   | 2015-2019     | China       | PCR                                | 90/878| 0.103         |
| Wei Z. (2020)       | 2016.2018.6   | China       | PCR                                | 11/621 | 0.018     |
| Couso-Pérez et al. (2020) | NA         | Portugal,Spain | IFAT                      | 10/79 | 0.127        |
| Wang (2020)         | 2016.2-2018.12 | China       | Microscopic examination             | 16/680 | 0.024       |
| Zhang (2021)        | 2018-2020     | China       | PCR                                | 9/590 | 0.015         |

NA, data is missing. PCR, Polymerase Chain Reaction; IFAT, International Federationt for Alternative Trade; DFA, Direct Immunoﬂuorescence Assay; ELISA, Enzyme-linked Immunosorbent Assay.
### TABLE 3 Summary of global equine Cryptosporidium infection rates.

| Variable          | Category               | No. studies | No. examined | No. positive | % (95% CI*) | Heterogeneity | Univariate meta-regression |
|-------------------|------------------------|-------------|--------------|--------------|-------------|---------------|-----------------------------|
|                   |                        |             |              |              |             | $\chi^2$                  | $P$-value | $I^2$ (%)  | $P$-value* | Coefficient (95% CI) |
| Detection methods | Molecular diagnostics  | 21          | 5982         | 430          | 7.49%       | (3.77-12.35)     | 603.44             | < 0.01   | 96.7     | 0.3555     | -0.0679(-0.2121 to 0.0762) |
|                   | Microscopic examination| 8           | 2843         | 327          | 10.52%      | (4.99-17.79)     | 200.33             | < 0.01   | 96.5     | 0.8605     | -0.0124(-0.1506 to 0.1256)   |
|                   | Immunological          | 7           | 1071         | 59           | 4.37%       | (1.83-7.95)      | 27.32              | < 0.01   | 78.0     | 0.0014     | -0.1792(-0.2889 to -0.0695)     |
| Gender            | Female                 | 12          | 1564         | 52           | 2.60%       | (0.78-5.44)      | 61.96             | < 0.01   | 82.2     | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
|                   | Male                   | 6           | 452          | 12           | 2.36%       | (0.04-8.07)      | 21.05              | < 0.01   | 76.2     | 0.5752     | -0.0584(-0.2628 to 0.1459)       |
|                   | ≥1year                 | 19          | 3257         | 394          | 11.06%      | (6.13-17.22)     | 346.09             | < 0.01   | 94.8     | 0.0014     | -0.1792(-0.2889 to -0.0695)     |
|                   | > 1year                | 16          | 3602         | 131          | 2.52%       | (0.97-4.77)      | 156.63             | < 0.01   | 90.4     | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
| Geographic area   | Asia                   | 14          | 5088         | 366          | 5.44%       | (2.24-9.94)      | 465.79             | < 0.01   | 97.2     | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
|                   | Oceania                | 1           | 67           | 12           | 17.91%      | (9.73-27.92)     | 0.00               | NA       | NA       | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
|                   | Europe                 | 9           | 1534         | 95           | 10.15%      | (4.77-17.24)     | 74.19              | < 0.01   | 89.2     | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
|                   | Africa                 | 2           | 481          | 11           | 2.27%       | (1.13-3.79)      | 0.30               | 0.58     | 0.00     | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
|                   | North America          | 4           | 746          | 60           | 7.37%       | (0.08-24.78)     | 69.68              | < 0.01   | 95.7     | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
|                   | South America          | 4           | 719          | 68           | 12.45%      | (2.84-27.51)     | 101.94             | < 0.01   | 97.1     | 0.1730     | 0.2025(-0.0888 to 0.4938)       |
| Sampling year     | Before 2008            | 9           | 1361         | 241          | 10.77%      | (3.99-20.32)     | 214.05             | < 0.01   | 96.3     | 0.0125     | -0.1615(-0.2883 to -0.0348)     |
|                   | 2009-2012              | 10          | 2580         | 306          | 10.73%      | (5.79-16.95)     | 200.18             | < 0.01   | 95.5     | 0.0125     | -0.1615(-0.2883 to -0.0348)     |
|                   | After or 2013          | 10          | 4419         | 181          | 3.04%       | (1.52-5.06)      | 114.57             | < 0.01   | 92.1     | 0.5752     | -0.0584(-0.2628 to 0.1459)       |
| Feeding method    | Cage-free              | 3           | 555          | 48           | 5.09%       | (0.00-20.00)     | 64.51              | < 0.01   | 96.9     | 0.5752     | -0.0584(-0.2628 to 0.1459)       |
|                   | Scale breeding         | 18          | 4995         | 546          | 7.86%       | (4.39-12.22)     | 500.57             | < 0.01   | 96.6     | 0.5752     | -0.0584(-0.2628 to 0.1459)       |
| Host              | Zebra                  | 1           | 10           | 1            | 10.00%      | (0.01-34.87)     | 0.00               | NA       | NA       | 0.6247     | 0.1419(-0.4267 to 0.7105)        |
|                   | Horse                  | 32          | 6851         | 480          | 7.32%       | (4.49-10.78)     | 684.18             | < 0.01   | 95.5     | 0.6247     | 0.1419(-0.4267 to 0.7105)        |
|                   | Male                   | 1           | 50           | 10           | 20.00%      | (10.20-32.09)    | 0.00               | NA       | NA       | 0.6247     | 0.1419(-0.4267 to 0.7105)        |
|                   | Donkey                 | 5           | 2906         | 333          | 7.52%       | (2.25-15.54)     | 196.58             | < 0.01   | 98.0     | 0.6247     | 0.1419(-0.4267 to 0.7105)        |
| Fraction          | middle                 | 21          | 3138         | 193          | 7.44%       | (4.05-11.74)     | 224.33             | < 0.01   | 91.1     | 0.0939     | 0.1839(-0.0313 to 0.3990)         |
|                   | high                   | 11          | 6410         | 575          | 5.93%       | (2.17-11.37)     | 606.89             | < 0.01   | 98.4     | 0.0939     | 0.1839(-0.0313 to 0.3990)         |
|                   | low                    | 3           | 269          | 48           | 17.36%      | (5.92-33.15)     | 18.76              | < 0.01   | 89.3     | 0.0939     | 0.1839(-0.0313 to 0.3990)         |
|                   | Total                  | 35          | 9817         | 816          | 7.59%       | (4.86-10.87)     |                      |          |          |           |                                    |

CI*: confidence interval; NA*: not applicable; P value*: P < 0.05 was statistically significant. Quality*: High: 5 points; Medium: 3 or 4; Low: 2.
probably due to a weaker body resistance of female Equus than males, especially after giving birth, and more susceptible to Cryptosporidium infection (Chen et al., 2013). The mare is considered to be a carrier of Cryptosporidium, and the young animals could be infected with Cryptosporidium by sharing a pasture or barn with the foals (Qi and Zhang, 2018). The eggs of Cryptosporidium in infected female Equus have no obvious shedding phenomenon, but the amount of shedding during the perinatal period increases (Skerrett and Holland, 2001).
This significantly increases the probability of young animals being infected with *Cryptosporidium*.

In this study, the prevalence of *Cryptosporidium* in *Equus* of ≤ 1 year was higher than that of *Equus* > 1 year, which is basically in line with the previous reports (Langkjaer et al., 2007; Wang, 2020). The maternal antibodies in the young animals will lose protective effects in 2-6 months, resulting in a decreased resistance of the young animals (Lu et al., 2008). In addition, the weaning stress response in young animals will lead to changes at hormone levels and immune function, thus causing immune system suppression, and thereby increasing the possibility of being infected with *Cryptosporidium* (Ren et al., 2018). Therefore, the breeding of dams and cubs needs to be strengthened to improve the resistance of animals, and the dams can be isolated during the breeding period.

Poor sanitary conditions increase the infection risk of *Cryptosporidium* in animals and expand the spread of
Cryptosporidium species (Gao et al., 2021). Our study of the rearing group showed that mules and donkeys had higher rates of Cryptosporidium infection than horses. The living environment of mules and donkeys is relatively complicated, and they usually suffer from poor harness, lack of veterinary care, improper nutrition, and low status and value, in spite of their usefulness (Davis, 2019). Meanwhile, the feces cannot be cleaned in time, which is more likely to cause the transmission and infection of Cryptosporidium in animals (Jian, 2012). An analysis of the breeding environment also showed that the
prevalence of Cryptosporidium was higher in Equus than that farmed on scale breeding. The high stocking densities of large-scale farming might result in high rates of Cryptosporidium infection in high-density captive equines. This may be due to the facts that more oocysts are scattered in high-density pens and the transmission speed is fast (Wang et al., 2008). Therefore, we recommend reducing stocking density and enhancing the animal welfare of donkeys and mules.

TABLE 4 Concentrated prevalence of equine Cryptosporidium in different countries.

| Variable  | Category | No. studies | No. examined | No. positive | % (95% CI*) |
|-----------|----------|-------------|--------------|--------------|-------------|
| Country   | Algeria  | 2           | 481          | 11           | 2.27% (1.13–3.79) |
| America   | 4        | 746         | 60           | 7.37% (0.08–24.78) |
| Belgium   | 1        | 134         | 6            | 4.48% (1.64–8.61) |
| Brazil    | 3        | 684         | 62           | 11.28% (0.76–31.64) |
| Canada    | 1        | 35          | 6            | 17.14% (6.67–31.19) |
| China     | 13       | 6122        | 562          | 5.65% (2.08–10.83) |
| Germany   | 2        | 51          | 4            | 4.84% (0.00–38.02) |
| Greece    | 1        | 190         | 2            | 1.05% (0.10–2.99) |
| Italy     | 3        | 260         | 40           | 19.58% (6.03–38.43) |
| Netherlands| 1      | 44          | 0            | 0.00% (0.00–2.17) |
| New Zealand| 1     | 67          | 12           | 17.91% (9.73–27.92) |
| Poland    | 2        | 424         | 21           | 5.81% (1.47–12.76) |
| Jordan    | 1        | 74          | 6            | 8.11% (3.03–15.36) |

TABLE 5 The prevalence of Cryptosporidium in different genotypes.

| Variable    | Category | No. studies | No. examined | No. positive | % (95% CI*) |
|-------------|----------|-------------|--------------|--------------|-------------|
| Genotype    | C. parvum| 17          | 348          | 80           | 31.95% (15.53–51.11) |
|             | C. andersoni | 5       | 34           | 12           | 38.83% (12.50–69.36) |
|             | C. hominis | 8        | 165          | 103          | 43.94% (23.11–65.95) |
|             | C. muris  | 2         | 19           | 11           | 53.55% (11.65–92.51) |
|             | Horse genotype | 10   | 245          | 41           | 29.24% (9.47–54.44) |
The investigation of sampling year in the selected articles showed that the prevalence of Cryptosporidium in Equus animals before 2012 was higher. In 2008, a worldwide economic crisis occurred and started to recover until 2010 (Zhang, 2018). The sluggish world economy may make people slack in Equus breeding, which may be one of the reasons for the high prevalence of Cryptosporidium. After 2013, the world economy gradually recovered, and many countries began to pay more attention to the animal husbandry. The gradually increased animal welfare may contributed to the lower prevalence of Cryptosporidium in Equus.

In the continent and country groups, the highest positive rate of Cryptosporidium was found in Oceania and the lowest was in Africa. Among them, New Zealand in Oceania had the highest positive rate, and Algeria in Africa had the lowest positive rate. New Zealand has a temperate maritime climate, with a warm and humid climate throughout the year, and its long coastline and abundant water resources make it more suitable for Cryptosporidium to survive. The frequent rainfall may lead to the transmission of Cryptosporidium in animal feces from fields to surface waters, thus resulting in a higher

### TABLE 6 A subgroup analysis of the prevalence of Cryptosporidium in equine genus by geographical location, climate and other variables.

| Variable       | Category       | No. studies | No. examined | No. positive | % (95% CI*) | Heterogeneity | Univariate meta-regression |
|----------------|----------------|-------------|--------------|--------------|-------------|---------------|---------------------------|
|                |                |             |              |              |             |               |                           |
| Latitude       | < 30°          | 7           | 794          | 45           | 8.77% (2.88-17.44) | 70.94 <0.01 | 91.5 | 0.1377 | -0.1346(-0.3122 to 0.0431) |
|                | 30°-35°        | 3           | 809          | 56           | 3.67% (0.02-13.11) | 60.19 <0.01 | 96.7 |
|                | 35°-40°        | 7           | 2357         | 213          | 2.70% (0.10-8.61) | 281.98 <0.01 | 97.9 |
|                | 40°-45°        | 7           | 1283         | 52           | 5.17% (0.74-13.23) | 70.21 <0.01 | 91.5 |
|                | > 45°          | 8           | 1597         | 121          | 7.45% (3.77-12.26) | 79.96 <0.01 | 91.2 |
| Longitude      | < 50°          | 7           | 1383         | 66           | 5.62% (1.27-12.79) | 76.48 <0.01 | 92.2 | 0.2230 | -0.1085 (-0.2830 to 0.0660) |
|                | 50°-100°       | 6           | 2108         | 39           | 1.75% (0.7-3.26) | 17.72 <0.01 | 71.8 |
|                | > 100°         | 9           | 3282         | 370          | 9.03% (3.15-17.53) | 305.79 <0.01 | 97.4 |
| Altitude (m)   | < 50           | 15          | 3717         | 372          | 6.88% (2.70-12.78) | 365.84 <0.01 | 96.2 | 0.2367 | -0.1096 (-0.2913 to 0.0720) |
|                | 50-100         | 6           | 858          | 21           | 2.41% (0.07-7.87) | 31.02 <0.01 | 83.9 |
|                | 100-150        | 8           | 1722         | 73           | 4.23% (1.08-9.32) | 72.01 <0.01 | 90.3 |
|                | > 150          | 6           | 543          | 21           | 4.94% (0.06-16.98) | 63.07 <0.01 | 92.1 |
| Rainfall (mm)  | < 500          | 3           | 620          | 20           | 1.71% (0.06-5.54) | 56.80 <0.01 | 84.2 | 0.0004 | -0.2751 (-0.4277 to 0.1225) |
|                | ≥ 500          | 2           | 1436         | 220          | 15.63% (8.60-24.28) | 117.90 <0.01 | 93.2 |
| Temperature (°C) | < 10          | 14          | 744          | 32           | 3.95% (0.34-11.25) | 97.30 <0.01 | 86.6 | 0.3395 | 0.0872 (-0.0918 to 0.2662) |
|                | ≥ 10           | 11          | 1599         | 217          | 8.04% (3.30-14.60) | 173.21 <0.01 | 94.2 |
| Humidity (%)   | 40-50          | 3           | 183          | 8            | 1.26% (0.00-8.59) | 6.95 0.03 | 71.2 |
|                | 50-60          | 10          | 581          | 33           | 4.41% (0.07-14.98) | 93.76 <0.01 | 90.4 |
|                | 60-70          | 8           | 661          | 50           | 5.02% (0.94-12.07) | 69.96 <0.01 | 90.0 |
|                | 70-80          | 3           | 569          | 142          | 22.69% (13.76-33.10) | 10.57 <0.01 | 81.1 |
| Climate        | Plateau alpine climate | 2 | 52 | 9 | 12.71% (0.00-78.20) | 26.62 <0.01 | 96.2 | 0.5059 | -0.0998 (-0.3937 to 0.1942) |
|                | Subtropical climate | 15 | 2797 | 255 | 7.82% (3.27-14.10) | 413.23 <0.01 | 96.6 |
|                | Temperate climate | 16 | 6251 | 455 | 5.81% (3.61-8.49) | 359.24 <0.01 | 94.7 |
|                | Tropical climate | 3 | 567 | 55 | 16.46% (0.48-48.11) | 123.02 <0.01 | 98.4 |

CI*, confidence interval; NA*, not applicable; P-value*: P < 0.05 is statistically significant.
prevalence of Cryptosporidium in this genus of horses (Sterk et al., 2016). In contrast, the Algerian coast has a Mediterranean climate, with high temperature and rainy winter, while the central and southern parts have a savanna and tropical desert climate, which is dry and rainy, with cold winter and hot summer. The increase of temperature will reduce the life activity of the worms on the land surface (Moriarty et al., 2012). Therefore, the prevalence of Cryptosporidium in Equus in Africa is low.

In the geographical groups, the positive rate of Cryptosporidium was the highest in regions with longitude > 100° and latitude < 30°. These two factors are mainly concentrated in the central and southern parts of China. The central and southern parts of China belong to the temperate monsoon climate and the subtropical monsoon climate. The rainfall types of these two climates are high temperature and rainy in summer, low temperature and less rain in winter (Wang, 2009). Meanwhile, the positive rate of Cryptosporidium in Equus that live in areas with humidity 70%-80%, rainfall > 500 mm, temperature ≥ 10°C, and altitude < 50 m was higher than that in other regions. High rainfall will increase the pollution of water sources, and Cryptosporidium is mainly transmitted through water sources, thereby increasing the prevalence of Cryptosporidium (Young et al., 2015). As altitude decreases, the temperature gradually increases (Zhu et al., 2013). Suitable temperature and hot and humid weather are the reasons for an increase in the prevalence of Cryptosporidium. This is in line with previous reports (Burton et al., 2010; Lv et al., 2021).

The genotypes of Cryptosporidium in infected Equus were analyzed. The main genotypes of Equus Cryptosporidium were C. parvum, horse genotype, C. andersoni, C. muris, C. hominis, and C. cuniculus (Wang, 2015; Wagnerova et al., 2016; Deng, 2018; Zhang, 2021). Among them, C. parvum, C. andersoni, and C. hominis are the genotypes that infect humans. In this study, the positive rates of C. muris and C. hominis genotypes were higher. Therefore, strengthening the feeding, management, and control of Equus Cryptosporidium are of great importance to the public safety of humans and animals.

The included articles mainly employed three types of detection methods, including molecular detection, immunological detection, and microscopic detection. Molecular examination is mainly based on PCR. Immunological tests include IFAT, DAF, and ELISA. Among them, the positive rate detected by IFAT technology is the highest, followed by microscopy and PCR. The microscopic detection of Cryptosporidium is easily operated and has a low cost but may lead to misdiagnosis and increase the probability of false positives (Robinson and Chalmers, 2020a). For immunological detection, although ELISA has the advantages of high sensitivity and specificity, it cannot perform species identification (Mittal et al., 2014). IFAT is prone to false positive, thus resulting in a high positive rate (Robinson et al., 2020b). The PCR detection method have high sensitivity and specificity than that of microscopy, has become the optimal method for Cryptosporidium detection (Zhang et al., 2017). It is recommended to use PCR method to detect Cryptosporidium in epidemiological investigations.

These results reflect the prevalence of Cryptosporidium in Equus to a certain extent around the world. In this meta-analysis, the reasons for losing points in some studies are: (1) less than 3 risk factors; (2) limited samples; (3) unclear sampling year; and (4) absence of specific sampling location. It is recommended that researchers should take more samples and explore more influencing factors to provide more data that support for the prevention and treatment of Cryptosporidium infection in Equus.

The advantages of this study are rigorous method and lots of risk factors. The publication bias was tested by using funnel chart and more accurate Egger’s test. A comprehensive analysis of subgroups can replenish the previous articles and provide more complete data for follow-up research. However, this study also has some limitations. First, we searched in five databases, and the search strategy might have caused the omission of some researches. Second, the data in some subgroups, such as Italy in the city subgroup, are covered only by one article, which can lead to unstable outcomes.

Concluding remarks

A systematic review and meta-analysis of 35 articles provided a comprehensive overview of the global epidemiology of Equus Cryptosporidium. The results showed that cryptosporidiosis was widespread in Equus, and cryptosporidiosis was prone to occur in the areas with warm climate. Equus under 1 year were more susceptible to Cryptosporidium infection. In view of the high incidence of cryptosporidiosis, correct prevention and control measures should be taken in time for specific age groups and regions. During the breeding process, good hygiene habits should be developed, regional prevention and control should be strengthened, and water pollution should be minimized.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the animal study.
Author contributions

X-ML and H-LG: Data curation, Methodology, Supervision, Writing-review and editing. W-LY and X-XZ: Writing-review and editing. Y-JW, Xin-YW: Data curation, Resources, Software. MZ and Xia-YW: Data curation, Methodology, Visualization. JL and GL: Conceptualization, Supervision, Funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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