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ORIGINAL PAPER

Large-scale survey of the prevalence of *Eimeria* infections in domestic rabbits in China

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Abstract The current study examined the prevalence of *Eimeria* infections in domestic rabbits in China. A total of 480 faecal samples were collected from 48 farms in 14 provinces of China. Each faecal sample was subjected to oocyst counting and oocyst isolation. The *Eimeria* species from samples containing isolated and sporulated oocysts were morphologically identified under microscope. The overall prevalence of infections was 41.9% (201/480). Northwest China had the highest prevalence (70%), followed closely by Northeast China (65%) and Southwest China (62.5%). The prevalences in North China (34%) and South China (25.8%) were significantly lower. The large and medium farms had lower prevalences (34.2% and 37.2%, respectively) than the small farms (61.4%). Coccidian oocysts were found in 42.2% (76/180) of faecal samples from meat rabbits, 40% (28/70) from angora rabbits and 44.7% (85/190) from Rex rabbit. In total, ten species of *Eimeria* were identified from oocyst-positive samples. Concurrent infection with two to eight *Eimeria* species was found. *E. perforans* was the most prevalent species (35.2%), followed in order by *E. media*, *E. magna*, *E. irresidua* and *E. intestinalis* with prevalences of 31.3%, 28.8%, 19.4%, and 14.8%, respectively. Taken together, These results reveal the characteristics of the prevelance of rabbit coccidia infection in China, including the distribution, the scale of farming and the species, which are indispensable to the control of rabbits coccidiosis in China.

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Introduction

China is the largest rabbit-producing country in the world. According to a report by the FAO, China produced approximately 700,000 t of rabbit meat in 2009, which constituted more than 40% of the world yield that year (Dalle Zotte and Szendro 2011). The rabbit industry in China is characterised by large number of breeds and uneven levels of managements, unlike the intensive breeding systems in Europe, which makes it very difficult to control infectious diseases in rabbits such as viral haemorrhagic disease and coccidiosis.

Rabbit coccidiosis is caused by 11 species of the genus *Eimeria*: *E. stiedai*, *E. magna*, *E. irresidua*, *E. flavescens*, *E. piriformis*, *E. intestinalis*, *E. exigua*, *E. perforans*, *E. vejdovskyi*, *E. coecicola* and *E. media* (Coudert 1989; Kvicerova et al. 2008; Licois 2009; Licois and Coudert 1982; Pakandl 2009). As one of the most common diseases in rabbits, coccidiosis results in severe economic losses to the industry every year (Varga 1982). All domestic rabbits can be infected by coccidia, especially the younger populations between 1 and 4 months of age (Pakandl et al. 2008). The morbidity and mortality in young rabbits can be as high as 90% and 60%, respectively (Meng et al. 2007).

In China, the prevalence of rabbit coccidiosis is complicated by the diversification of the industry. For example, there are many breeds, including Rex rabbits, meat rabbits and angora rabbits, and the farming scale ranges from scattered farms to intensive farms. Previous surveys have shown that *Eimeria* infection in rabbits is

common in some provinces of China (Chen et al. 1997; Li et al. 1998; Meng et al. 2007; Zhao et al. 2008). However, there is limited information available on the presence of coccidia species in rabbits in China. Here, we report our country-wide epidemiological survey (five areas consisting of 14 provinces) of the prevalence of rabbit coccidiosis in China, which will facilitate the establishment of an efficient control system for rabbit coccidiosis in China.

Materials and methods

Study area

Based on the combined factors affecting the rabbit industry, including the distribution of farms, the influence of the climate, the breeding scale, the level of management and the rabbit breeds on farms in China, we choose areas and provinces possessing large numbers of rabbits, such as North China (Hebei, Shandong, Henan and Shanxi Provinces), South China (Zhejiang, Jiangsu, Fujian, Anhui and Guangdong Provinces), Southwest China (Sichuan and Yunnan Provinces), Northwest China (Inner Mongolia) and Northeast China (Jilin and Liaoning Provinces). All chosen areas and provinces are shown in Fig. 1.

Rabbit populations for sampling

Faeces were collected from 480 rabbit populations, which were from 48 farms in the 14 provinces described above, and were tested for the presence of oocysts. These rabbits included 190 populations of Rex rabbits, 70 populations of angora rabbits and 180 populations of meat rabbits. There were 204 populations of weanling rabbits (1 to 3 months old), 109 populations of young rabbits (3 to 6 months old) and 147 populations of adult rabbits (older than 6 months); and there were 120 populations from large-scale farms (10,000 or more rabbits on hand at the farm), 290 populations from medium-scale farms (between 1,000 and 10,000 rabbits) and 70 populations from small-scale farms (fewer than 1,000 rabbits).

Fig. 1 Study areas for the survey of the prevalence of rabbit *Eimeria* infection in China. All provinces involved are marked with a *star*

Table 1 Prevalence and intensity of coccidia infection in different regions of China

Locality	Examined no.	Positive no.	Prevalence (%)	OPG
North China	200	68	34.0	77,090
South China	120	31	25.8	4,926
Southwest China	120	75	62.5	80,556
Northwest China	20	14	70.0	29,726
Northeast China	20	13	65.0	18,978

Sampling and treatment method

From July to October, 2010, trained senior students from the College of Veterinary Medicine, China Agricultural University, were dispatched to the above-mentioned areas for sample collection. From each chosen population, 500 g of fresh faecal pellets were collected as one sample. The faeces were stored in ziplock bags and delivered to the laboratory by express mail service, which took 2 to 4 days. After arrival, all samples were stored at 4°C until analysis. Each faecal sample was homogenised in 500 ml tap water, and then 2 g of the mixture was put into 60 ml of saturated salt solution (Carvalho et al. 2011; Mundt et al. 2005; Velkers, et al. 2010). The suspension was then emptied into a modified McMaster chamber (Coudert et al. 1995). Five

Table 2 Percentage of faecal samples infected with each coccidia species in rabbits in China

Species	Examined no.	Positive no.	Prevalence (%)	OPG
E. magna	480	138	28.8	43,400
E. media	480	150	31.3	17,200
E. coecicola	480	28	5.83	132,400
E. intestinalis	480	71	14.8	41,400
E. perforans	480	169	35.2	40,200
E. irresidua	480	93	19.4	9,200
E. piriformis	480	13	2.71	3,200
E. flavescens	480	22	4.58	9,600
E. stiedai	480	2	0.42	1,400
E. exigua	480	1	0.002	2,800

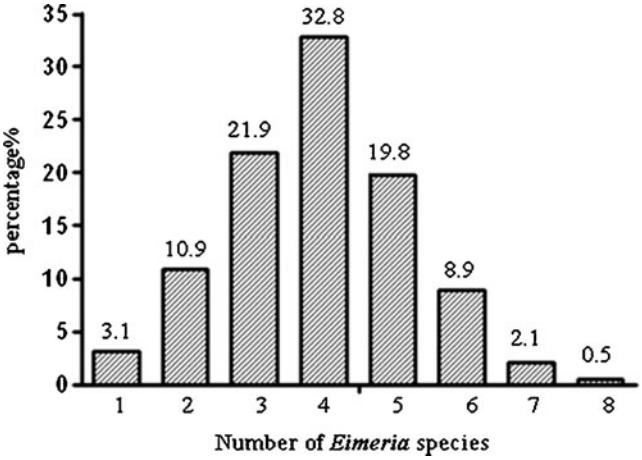

Fig. 2 Percentages of single and mixed infections with different *Eimeria* species in rabbits in China

minutes later, the oocysts within the chamber were counted under the microscope and the oocyst per gram (OPG) was calculated to estimate the degree of infection. The limitation of detection is 200 oocysts per gram facal sample.

Species identification

Oocysts were purified as previously described (Coudert et al. 1995; Kvicerova et al. 2008) and were sporulated by shaking in a 2.5% potassium dichromate solution at 28°C for 7 days to ensure good aeration, and stored at 4°C until morphological identification. Concentrated oocysts in each sample were identified based on their sizes and morphological characteristics (shape, colour, form index, presence or absence of the micropyle and its cap, presence or absence of residual, polar and Stieda bodies) of the oocysts and sporocysts (Coudert et al. 1995; Kvicerova et al. 2008). To ensure that species identification is valid, at least 50 sporulated oocysts from each species were observed and measured.

Statistical analysis

The statistical package SPSS was used for data analyses, and a value of $P<0.05$ was considered significant.

Results

Overview of the prevalence of coccidian infection in rabbits in China

A total of 480 samples were collected and analysed: 200 from North China, 120 from South China, 120 from Southwest China, 20 from Northwest China and 20 from Northeast China. Overall, coccidian oocysts of *Eimeria* were found in 201 of 480 faecal samples (41.9%) obtained from the fourteen provinces. Northwest China had the highest prevalence (70%) of positive samples, followed closely by Northeast China (65%) and Nouthwest China (62.5%). The prevalence in North China (34%) and South China (25.8%), were significantly lower (Table 1).

The morphological identification of *Eimeria* oocysts revealed the presence of ten species of *Eimeria*, namely, *E. stiedai*, *E. magna*, *E irresidua*, *E. media*, *E piriformis*, *E. intestinalis*, *E. flavescens*, *E. exigua*, *E. coecicola*, and *E. perforans*. *E. perforans* was the most prevalent species (35.2%), followed in order by *E. media*, *E. magna*, *E. irresidua* and *E. intestinalis* with prevalences of 31.3%, 28.8%, 19.4%, and 14.8% respectively (Table 2).

Concurrent infections of *Eimeria* in domestic rabbits in China

Concurrent infections with more than one *Eimeria* species were commonly present in rabbits. Most of the rabbits carried two to eight species. *E. perforans*, *E. media* and *E. magna* were the species most frequently found in concur-

Table 3 Percentage of faecal samples infected with each coccidia species in rabbits in China

	North China	South China	Southwest China	Northwest China	Northeast China
Species	(n=200)	(n=120)	(n=120)	(n=20)	(n=20)
E. magna	54/27.0	19/15.8	42/35	13/65.0	10/50.0
E. media	54/27.0	27/22.5	45/37.5	13/65.0	10/50.0
E. coecicola	11/5.5	1/0.83	27/22.5	3/15.0	4/20.0
E. intestinalis	18/9.0	16/13.3	53/44.17	3/15.0	7/35.0
E. perforans	56/28.0	30/25.0	38/31.67	14/70.0	12/60.0
E. irresidua	24/12.0	18/15.0	16/13.33	8/40.0	5/25.0
E. piriformis	4/5.0	1/0.83	7/5.83	0/0	1/5.0
E. flavescens	5/4.0	5/4.17	1/0.83	3/15.0	2/10.0
E. stiedai	1/0.5	0/0	1/0.83	0/0	0/0
E exigua	0/0	0/0	1/0.83	0/0	0/0

Table 4 Prevalence and intensity of coccidia infection in different rabbit breeds in China

Breed	Examined no.	Positive no.	Prevalence (%)	OPG
Meat rabbit	180	76	42.2	14,842
Angora rabbit	70	28	40.0	22,258
Rex rabbit	190	85	44.7	83,690

rent infections. The percentages of single and mixed infections of different *Eimeria* species in rabbits are shown in Fig. 2.

Prevalence of coccidian infection of rabbits in five areas of China

In North China, *E. exigua* was not detected. *E. perforans* was found to be the most prevalent (28%) species, followed in order by *E. magna* (27%), *E. media* (27%) and *E. irresidua* (12%). In South China, *E. exigua* and *E. stiedai* were not detected, and the most common species were *E. perforans* (25%), *E. media* (22.5%), *E. magna* (15.8%), *E. irresidua* (15%) and *E. intestinalis* (13.3%). In Southwest China, all ten species were detected, and *E. intestinalis* was the most prevalent (44.2%) species, followed in order by *E. media* (37.5%), *E. magna* (35%), *E. perforans* (31.7%), and *E. coecicola* (22.5%). In Northwest China, three species, *E. piriformis*, *E. exigua* and *E. stieda* were not detected. *E. perforans*, *E. magna*, *E. media* and *E. irresidua* were the four most common species, with prevalences of 70%, 65%, 65% and 40%. In Northeast China, *E. exigua* and *E. stiedai* were not detected, and the most common species were *E. perforans* (60%), *E. media* (50%), *E. magna* (50%), and *E. intestinalis* (35%) (Table 3).

Influence of breed, age, farm scale and anticoccidials usage on the prevalence of *Eimeria* infection in rabbits

Coccidian oocysts were found in 42.2% (76/180) of faecal samples from meat rabbits, 40.0% (28/70) from angora rabbits, and 44.7% (85/190) from Rex rabbits; these values were not significantly different ($P>0.05$) (Table 4). Rex rabbits had the highest OPG (83690), while meat rabbits had the lowest OPG (14842).

Table 5 Prevalence and intensity of coccidia infection in adult and young animals in China

Age class	Examined no.	Positive no.	Prevalence (%)	OPG
Breeding rabbits	147	41	27.9	7,138
Growing rabbits	109	35	32.1	14,252
Kits of rabbits	204	105	51.5	84,050

The prevalence of coccidian oocysts in young rabbits (51.5%) was higher than in growing rabbits (32. 1%) and breeding rabbits (27.9%), and the differences were statistically significant ($P<0.05$) (Table 5). The intensity of infection in young animals was also significantly higher ($P<0.05$) than in adults, which is consistent with previous observations (Pakandl et al. 2008).

Table 6 Prevalence and intensity of coccidia infection on different types of farms

Types of farms (no. of rabbits on a farm)	Examined no.	Positive no.	Prevalence (%)	OPG
Large (>10,000)	120	41	34.2	7,506
Medium (1,000–10,000)	290	108	37.2	61,090
Small (<1,000)	70	43	61.4	23,514

As shown in Table 6, the large and medium farms had lower prevalences of coccidian oocysts than the small farms. Coccidian oocysts were found in 34.2% (41/120) of the faecal samples from large farms, 37.2% (108/290) from medium farms, and 61.4% (43/70) from small farms.

All of the 48 farms used anticoccidials to their rabbits for coccidiosis prevention, while 11 farms did not provide us the kinds of anticoccidials they used. Diclazuril, robenidine and sulfonamides are the most frequently used drugs in the farms. In farms using diclazuril, coccidian oocysts were found in 43.8% (57/130) of faecal samples compared to 32.5% (13/40) for those using robenidine and 42.5% (17/40) for those using sulfonamides, respectively. Two or three anticoccidials were used in 43.2% (16/37) farms. Combination of two drugs could not lower the prevalence of coccidian oocysts in the farms (Table 7),while the application of three drugs can significantly reduce the prevalence

Table 7 Prevalence and intensity of coccidia infection on farms with different anticoccidials useage in China

Anticoccidials	Examined no.	Positive no.	Prevalence (%)	OPG
Dicla	130	57	43.8	57,400
Robe	40	13	32.5	34,800
Sulfo	40	17	42.5	28,000
Dicla+Robe	80	34	42.5	35,000
Dicla+Sulfo	20	12	60.0	45,200
Dicla+Sali	20	6	30.0	29,000
Robe+Sulfo	10	4	40.0	32,600
Dicla+Robe+Sulfo	30	5	16.7	13,600
Unknown	110	54	49.1	42,400

Dicla diclazuril, *Robe* robenidine, *Sulfo* sulfonamides, *Sali* salinomycin

and the intensity of the infection in farms ($P<0.05$) (Table 7). Those farms (11/48) employing unknown anticoccidials had a higher coccidian prevalence (49.1%).

Discussion

In the present study, the prevalence of coccidia infection in China was surveyed. Based on the analysis of 480 faecal samples collected from 48 rabbit farms distributed in five areas of China, the overall infection rate was 41.9%. The existence of ten *Eimeria* species was confirmed in the oocyst-positive samples.

A previous report showed that the prevalence rate of coccidiosis in commercial rabbit farms in China was approximately 60% (LI 1999). The lower prevalence of 41.9% in the current study reveals that the control of rabbit coccidiosis has improved country-wide, especially in those farms with a large or medium number of rabbits (Table 6). The reason for this improvement is that commercial feeds containing anticoccidial drugs have become much easier to obtain in larger farms (Pan et al. 2008). However, in small farms, grass, silage and grain are widely used as rabbit feed, making the administration of anticoccidials in feed impracticable, although practice other than anticoccidials was also employed in rabbit farming, poor hygienic conditions and suboptimal temperatures were observed on some small individual rabbit farms, which can favour the occurrence of *Eimeria* spp. infections.

E. stiedai, *E. magna*, *E. irresidua*, *E. flavescens*, *E. piriformis* and *E. intestinalis* are regarded as pathogenic in rabbits (Polozowski 1993). Although *E. magna*, *E. irresidua* and *E. intestinalis* were the dominant species in the examined faecal samples, most of the OPG values for these samples were far less than those corresponding to clinical coccidiosis. This result indicates that sub-clinical coccidiosis is common in the rabbit industry. Sub-clinically infected rabbits appear normal outwardly, but may suffer from reduced feed consumption, feed conversion and growth performance, resulting in huge economical losses for this industry.

The influences of age and breeds and anticoccidials usage were also taken into account in the current study. Lower resistance or less immunity to coccidian infection in young rabbits than in older animals (Pakandl et al. 2008) is responsible for the high prevalence of coccidiosis in young rabbits (51.5%), but the prevalence of coccidian oocysts in meat rabbits, angora rabbits and Rex rabbits was not statistically significantly different ($P>0.05$), which suggests that the breed of rabbit has little effect on the prevalence of coccidiosis in domestic rabbits. In addition, the prevalence of coccidian oocysts in most farms employing different anticoccidial drugs shows no significantly differentiation, suggesting that *Eimeria* species of rabbits in China have developed drug resistance due to the lack of standardization.

McMaster technique has a cutoff value based on the amount of feces and volume of flotation solution, the sensitivity of the test used in this study is 200 oocysts per gram of sample as we only counted oocysts in one chamber. As a result, OPG was less than that which could not be detected by this technique, which is particularly so in small herds, when animals are excreting a low number of eggs (Levecke et al. 2011). So we might have underestimated the actual prevalence based on this technique. Combination with other molecular diagnostic assays such as PCR should be applied to improve sensitivity of detection in future.

Overall, although more factors relevant to the epidemiological study of rabbit coccidiosis such as the season should be included in further studies, this study revealed the characteristics of the prevalence of rabbit coccidial infection in China and provided relevant "baseline" data for assessing the effectiveness of future control strategies against rabbit coccidiosis in China.

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