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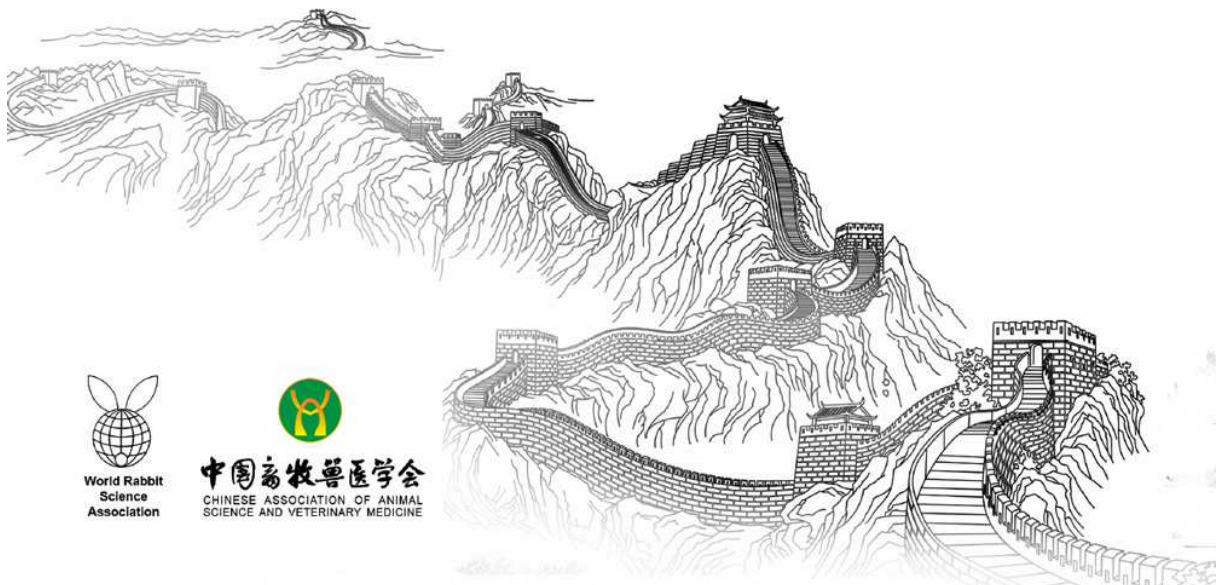
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PREVALENCE OF COCCIDIOSIS IN DOMESTIC RABBITS IN THE THREE GORGES RESERVOIR AREA OF CHINA

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ABSTRACT

The present study is the first extensive epidemiological investigation of coccidial infection in domestic rabbits in the Three Gorges Reservoir Area (TGRA) of China. The study was conducted to investigate the prevalence of coccidiosis and most prevalent species of *Eimeria* in domestic rabbits. A total of 3,169 faecal samples of domestic rabbits were randomly collected from 213 rabbit farms in 19 riparian counties in the TGRA from September 2012 to June 2014. Oocyst per gram of faeces (OPG) were counted using the McMaster method. The prevalent species of *Eimeria* were morphologically identified by microscope. The overall prevalence of coccidiosis in the TGRA was 77.06% which ranged from 66.91%-86.84%. Mixed infections with two or more *Eimeria* spp. were found in all positive fecal samples. In total, nine different *Eimeria* species were identified from positive samples, with *E. irrestidua* (43.22%, 1370/3169) being the most prevalent specie in the TGRA. Coccidia infection were found in weanling rabbits (1~3 months old) more frequently than in young rabbits (3~6 months old) and adult rabbits (>6 months old) ($P<0.01$). The results indicate the prevalence rate of coccidial infection is appeared to be higher than previous reports in other areas of China. This needs effective preventive measures in time to control any major outbreak on rabbit farms.

Key words: Coccidiosis, three Gorges Reservoir Area (TGRA), Oocyst per gram faeces (OPG)

INTRODUCTION

The Three Gorges Dam (TGD) is one of the largest hydraulic power complex in the world. This has also created the Three Gorges Reservoir Area (TGRA) with 79,000 square kilometers stretching from Yichang City to Chongqing between 106°50'-110°50' E and 29°16'-31°25' N, and including 19 riparian counties (Yichang, Zigui, Xingshan, Badong, Wushang, Wuxi, Fengjie, Yunyang, Kaixian, Wanzhou, Zhongxian, Shizhu, Fengdu, Wulong, Fuling, Changshou, Yubei, Banan, and Jiangjing) in the Yangtze valley (Chau 1995; Gao Qun and Mao Hanying 2007; Jackson and Sleight 2000). Within the TGRA, 95% of the area consists of hills and mountains and only 4.3% is flatland. Since 1993, the rabbitry is one of the most important industries in Agricultural economy in the reservoir area. According to the latest statistics, a total of 44.8 million rabbits are feed in the TGRA in 2012.

Coccidiosis caused by protozoans of *Eimeria* genus (Apicomplexa: Eimeriidae) is one the most prevalent condition on the rabbit farms around the world (Bhat et al., 1996). This disease can not only lead to retarded growth, diarrhea and mortality but also facilitates other enteric diseases such as colibacillosis, which causes serious economic losses in the TGRA since 2012 to 2014. Recent reports have shown that the prevalence of coccidial infection is 41.9% in domestic rabbits in China (Fa Jing et al., 2011). However, the epidemiological date about coccidial infection in domestic rabbits is very limited in this region. Therefore, we investigated the prevalence of coccidial infection in the TGRA in the past three years. This is the first extensive survey of the prevalence of coccidiosis in domestic rabbits in the TGRA. The goal of this survey was to determine the most prevalent species and prevalence of coccidial infection in domestic rabbits. The data from this survey

will assist in the development of effective prevention and control program against coccidiosis of rabbit.



Fig.1. A map of the TGRA with evidence of surveyed regions. All counties involved are marked with a round dot.

MATERIALS AND METHODS

Faecal samples collection

A total of 3,169 faecal samples of domestic rabbits were collected from 213 rural rabbit farms in 19 riparian counties in the TGRA between September 2012 to June 2014 (Fig 1). Of the 213 rabbit farms, 143 farms used robenidine in rabbit feed as a prophylactic against coccidiosis; 48 used diaveridine; 12 used sulfachloropyrazine sodium; and 10 farms did not use any coccidiostats. rabbit population was approximately 3,000,000 in these rabbit farms. These rabbits belonged to different age groups including weanling (1~3 months old), young (3 ~ 6 month old) and adult rabbits (>6 months old). For sampling, 100g of fresh faeces were collected and stored in a sterile homogeneous bag. All the samples were marked with the sampling sites, date, and then transferred to parasitological laboratory.

Oocyst per gram of faeces (OPG) measurement

The accurate weight of each fecal sample was determined in a 1,000 ml clean beaker. Then, 100 g of fecal sample was mixed into 200 ml tap water, homogenized by vigorous stirring at room temperature, and later 10 ml of NaCl solution was added in 1g of the mixture. The number of oocysts in the fecal sample was counted by the McMaster method (Gerhold et al., 2011b). The limitation of detection was 200 oocysts per gram of fecal sample.

Oocyst per gram faeces (OPG) was classified to three levels according to the diagnostic manual for animal coccidiosis of National Standards of the People's Republic of China: Level 1: severe infections ($OPG > 10 \times 10^4$); Level 2: moderate infections ($1 \times 10^4 < OPG \leq 10 \times 10^4$); Level 3: mild infections ($OPG \leq 1 \times 10^4$).

Prevalent species of *Eimeria* identification

Oocysts of *Eimeria* spp were purified by the flotation method using saturated NaCl solution. The oocysts were sporulated by shaking in a 2.5% potassium dichromate solution at room temperature (28°C) for 1 week. Later these oocysts were identified based on morphological characteristics of oocysts (shape, size, color, presence or absence of micropyle and its cap).

Date analysis

The χ^2 -test was used for statistical association by using SPSS statistical software version 14. A value of $p < 0.05$ was considered as significant.

RESULTS

The prevalence of coccidian infection in domestic rabbits

Among all faecal samples, *Eimeria* oocysts were found in 2442 (77.06%, 2442/3169) samples, and prevalence rate of coccidiosis was ranged from 66.91%-86.84% in all counties. The highest prevalence was recorded in Fengjie country (86.84%) while Wanzhou country had lowest prevalence of coccidiosis (66.91%; Table 1).

Table 1 Positive rate of coccidia infection in domestic rabbits in TGRA

counties	No. farms	No. samples	No. positive samples	Prevalence (%)
Yichang	10	156	118	75.64 (118/156)
Zigui	9	137	105	76.64 (105/137)
Xingshan	9	133	96	72.18 (96/133)
Badong	9	142	105	73.94 (105/142)
Wushan	10	150	118	78.67 (118/150)
Wuxi	12	161	120	74.53 (120/161)
Fengjie	12	152	132	86.84 (132/152)
Yunyang	11	163	117	71.77 (117/163)
Wanzhou	8	139	93	66.91 (93/139)
Kaixian	14	236	198	86.84 (198/228)
Zhongxian	12	175	138	78.86 (138/175)
Shizhu	16	289	241	83.39 (241/289)
Fengdu	12	145	109	75.17 (109/145)
Wulong	10	142	102	71.83 (102/142)
Fuling	12	150	115	76.67 (115/150)
Changshou	13	202	166	82.18 (166/202)
Yubei	11	158	116	73.42 (116/158)
Banan	11	166	120	72.29 (120/166)
Jiangjing	12	173	133	76.88 (133/173)
Total	213	3169	2442	77.06 (2442/3169)

Species identification and mixed infections of *Eimeria* in domestic rabbits

Mixed infection with two or more *Eimeria* spp. was found in all positive fecal samples (100%, 2444/2444). Nine different *Eimeria* species were identified and their prevalence rate is as follow: *E. irrestidua* (43.22%, 1370/3169), *E. perforans* (21.14%; 670/3169), *E. intestinalis* (18.37%; 582/3169), *E. magna* (15.37%; 487/3169), *E. stiedai* (13.83%; 438/3169), *E. flavescens* (13.00%; 412/3169), *E. coecicola* (11.83%; 375/3169), *E. neleporis* (9.69%; 307/3169) and *E. exigua* (9.15% ; 290/3169). *E. irrestidua* (43.22%) was the dominant species of *Eimeria* in the TGRA (Table 2).

Table 2: Positive rate of 9 *Eimeria* spp. in domestic rabbits in TGRA

<i>Eimeria</i> sp.	No. of positive	Prevalence (%)
<i>E.irrestidua</i>	1370	43.22 (1370/3169)
<i>E.perforans</i>	670	21.14 (670/3169)
<i>E.intestinalis</i>	582	18.37 (582/3169)
<i>E.magna</i>	487	15.37 (487/3169)
<i>E.stiedae</i>	438	13.83 (438/3169)
<i>E.flavescens</i>	412	13.00 (412/3169)
<i>E.coecicola</i>	375	11.83 (375/3169)
<i>E.neleporis</i>	307	9.69 (307/3169)
<i>E.exigua</i>	290	9.15 (290/3169)

Influence of age on the prevalence of *Eimeria* infection in domestic rabbits

Age is one of the most principal factors in coccidiosis (Pakandl et al., 2008). In our study, the prevalence rate of coccidiosis was higher (85.6%; $P < 0.05$) in weanling rabbits than young (76.27%) or adult rabbits (59.90%) (Table 3). A significantly higher number of OPG ($> 1 \times 10^4$) was recovered ($P < 0.01$) in weanling rabbits than adult rabbits, whereas, lower OPG ($< 1 \times 10^4$) was found in adult rabbits (59.03%) than from young (72.84%)or weanling rabbits (78.14%). (Table 3).

Table 3: OPG in domestic rabbits at different ages

counties	Ages	No. samples	A	a(%)	B	b(%)	C	c(%)	Prevalence (%)
Total	1 ~3 months	1464	1144	78.14	78	5.33	32	2.19	85.66(1254/1464)
	3 ~6 months	902	657	72.84	24	2.66	7	0.78	76.27 (688/902)
	>6 months	803	474	59.03	5	0.62	2	0.25	59.90 (481/803)

a: $OPG \leq 1 \times 10^4$ b: $1 \times 10^4 < OPG \leq 10 \times 10^4$ c: $OPG > 10 \times 10^4$
 a(%):Prevalence of A b(%):Prevalence of B C(%):Prevalence of C

DISCUSSION AND CONCLUSION

Coccidiosis, caused by *Eimeria* species is one of the major disease problems for rabbit producers. This economic importance of this disease has been well reported in livestock species (Rehman et al., 2011). Similarly, its prevalence rate and dominant coccidial species were reported in different geographical areas (Jithendran et al., 1996; Meng et al., 2007; Qiao et al., 2008 and 2012; Razavi et al., 2010; Fa Jing et al., 2011; P.O.Okumu et al., 2014). In this study, a comprehensive survey of coccidial infection in domestic rabbits of 19 different riparian counties was carried out. The overall prevalence rate was 77.06% in our study which is higher than earlier reported by Qiao et al. (2012) and Fa et al. (2011) in China. The most probable reason of this increase is that a large number of small-scale rabbit farms exist in these counties. Furthermore, poor hygienic conditions, suboptimal temperatures and humidity were also observed in small-scale rabbit farms which favor the occurrence of *Eimeria* spp. infection. In addition, *Eimeria* species in most small-scale farms of the TGRA have developed drug resistance due to long-term use of a single anticoccidial drug.

A previous study by Fa et al. (2011) reported 10 *Eimeria* spp. with higher incidence of *E. perforans* (35.2%), *E. Magna* (28.8%) and *E. Media* (31.3%), while another study by Qiao et al. (2012) detected 14 *Eimeria* spp. in domestic rabbits with higher incidence of *E. irrestidua* (23.98%), *E. perforans* (19.61%) and *E. stiedae* (17.81%). In contrast, our study reported higher incidence of *E. irrestidua*, *E. perforans*, *E. intestinalis* and *E. magna* were the dominant species separated in the TGRA. These results indicated that the dominant species of *Eimeria* were different in different geographic areas of China. Our results showed that maximum prevalence of coccidiosis was found in weanling rabbits (1~3 months old) which is consistent with the findings of Qiao et al. (2012), Fa et al. (2011) and Pakandl et al. (2008).

In conclusion, this present report revealed the prevalence of coccidial infection in domestic rabbits in the TGRA and provided a large number of epidemiological data for development of effective prevention and control strategies against rabbit coccidiosis in China.

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