J Parasit Dis (Oct-Dec 2017) 41(4):1014–1019
DOI 10.1007/s12639-017-0927-1

ORIGINAL ARTICLE

Prevalence of *Eimeria* species in domestic chickens in Anhui province, China

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Received: 16 February 2017 / Accepted: 12 May 2017 / Published online: 22 May 2017

Abstract Prevalence studies can adequately assist in the design of prophylaxis strategies for disease control. Here, the prevalence of *Eimeria* species in chickens was investigated in Anhui province, China, from July to September 2016. A total of 171 samples were tested by microscopic examination and molecular methods. The prevalence of coccidiosis in Anhui province was found to be 87.75% (150/171). *Eimeria tenella* was the most prevalent species (80.67%, 121/150), and *Eimeria necatrix*, *Eimeria mitis*, *Eimeria maxima*, *Eimeria brunetti* and *Eimeria acervulina* were 68% (102/150), 55.33% (83/150), 54.67% (82/150), 44.67% (67/150) and 2.67% (4/150), respectively. *Eimeria praecox* was not detected at all. The most common combinations are *E. tenella*, *E. maxima*, *E. necatrix*, *E. brunetti* and *E. mitis* (26.67%, 40/150), followed by *E. tenella*, *E. maxima* and *E. necatrix* (19.33%, 29/150). *Eimeria necatrix* exhibited the highest participation in multiple infections. The results of the present study suggested that *Eimeria* infection is mixed, severe and widespread in chickens, Therefore, integrated strategies should be performed to prevent and control coccidial infection in chickens in Anhui province.

Keywords Prevalence · Domestic chickens · *Eimeria* species · Molecular methods · Identification

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Introduction

Coccidiosis is caused by the protozoan parasite of the genus *Eimeria* spp. consisting of seven species, and is one of the most prominent problems faced by the modern poultry industry (Vrba et al. 2011). Annual global poultry production losses are consequently large and are estimated at billions of dollars (Godwin and Morgan 2014). The morbidity and mortality in chickens infected with *Eimeria* species can be as high as 80% (Mcdougald et al. 1997). Therefore, coccidiosis is considered to be one of the most costly diseases (Zaman et al. 2012). In the poultry industry, chickens can be infected by any of the seven *Eimeria* species, including *Eimeria tenella* (*E. tenella*), *Eimeria maxima* (*E. maxima*), *Eimeria acervulina* (*E. acervulina*), *Eimeria necatrix* (*E. necatrix*), *Eimeria brunette* (*E. brunette*), *Eimeria mitis* (*E. mitis*), and *Eimeria praecox* (*E. praecox*) (Shivaramaiah et al. 2014). *E. tenella*, *E. necatrix*, *E. maxima*, *E. brunette* and *E. acervulina* are considered to be highly pathogenic, whereas *E. praecox* and *E. mitis* are regarded as the least pathogenic (Al-Natour et al. 2002).

China is one of the three largest chicken-producing country, ranked third in exports, behind the United States and Brazil (USDA 2015). In China, the poultry industry has an important economic status throughout the entire country. The chicken industry in China is characterized by a large number of breeds and irregular managements, in contrast to the intensive breeding systems in Europe. This makes it extremely difficult to control infectious diseases in chickens such as coccidiosis. Annual economic losses due to coccidiosis were more than 73 million USD, with the drug costs of poultry producers ranging from 73 to 88 million USD. Of the total combined pharmaceutical control costs of all potential chicken diseases, coccidiosis alone

accounts for 30% of the total cost (Suo 2004). Anhui is a chicken-producing province of China. However, chicken coccidiosis is one of the main diseases endangering the chicken industry due to the high morbidity and mortality rate. Therefore, this disease causes tremendous economic losses to the chicken industry very year, and is severely affected by frequent outbreaks of diseases (Shang et al. 2011).

In the face of challenges posed by coccidiosis, several clinical and epidemiological studies have been undertaken in recent years, including those in the provinces of Shandong and Heilongjiang (Sun et al. 2009; Li et al. 2009). To date, seven *Eimeria* species have been reported to infect chickens in China (Sun et al. 2009). Confirm whether this hypothesis is still applicable to the present condition. The purpose of the present study was to analyze the epidemiology of *Eimeria* species in Anhui province using molecular methods based on ITS-1 rDNA regions. The specific identification of *Eimeria* species is important for the diagnosis, disease control, and treatment, as well as for epidemiology and biological studies of chicken populations (Hamza et al. 2015). This data will provide critical information regarding the epidemiology of coccidian infection among domestic chickens in Anhui province.

Materials and methods

Sample collection

Fresh chicken faeces (20–45 days) were collected from 171 farms located in Feixi county (31 farms), Feidong county (28 farms), Changfeng county (32 farms), Lujiang county (31 farms), Fengtai county (28 farms) and Quanjiao county (21 farms), from July to September 2016. The breeding stock of each farm was between 5000 and 10,000 chickens. Fresh faeces were collected from the four corners and center of each chicken house in a plastic bag and homogenized thoroughly. All samples were kept in a refrigerator at 4 °C until further used.

Laboratory processing

A total of 2 g of each sample was put into 60 mL of a saturated salt solution. The suspension was filtered through gauze and centrifuged at 1500 rpm for 10 min. A wire ring was then used to obtain a sample of the liquid film for microscopic examination with light microscopy. The oocyst-positive samples were homogenized in 0.75% (w/v) physiological saline until it could be washed by 0.75% (w/v) physiological saline through a sieve covered with folded gauze into a beaker. The solids components of the filtrate were permitted to settle out by centrifugation at 3500 rpm for 10 min. The supernatant was discarded and the sediment was purified twice in a saturated saline solution by centrifugation at 1500 rpm for 10 min. The sediment was discarded and the supernatant was washed twice by centrifugation at 3500 rpm for 10 min with tenfold distilled water. The sediment containing oocysts was incubated in 2.5% (w/v) potassium dichromate on petri dishes to sporulation at 27 °C for 5 days. The sporulated oocysts were stored at 4 °C.

Microscopic examination

Oocysts from each sample were photographed using a microscope coupled Motic Images Plus 2.0ML digital camera (Motic BA 210, Motic company, Xiamen, China) and then measured for size. The morphological parameters were detected by indexes for length, width and shape.

DNA extraction

The oocysts were washed with distilled water to remove the potassium dichromate. Using 0.5 mm diameter glass beads, the samples were vortexed vigorously to rupture the walls of the oocysts. The DNA was extracted using the E.Z.N.A.Micro Elute Genomic DNA Kit (Omega, America), following the manufacturers protocol with minor modifications. We used 300 µL of the sporulated oocysts suspension, instead of feces and the vortex time was 1 min instead of 30 s. The DNA concentration was determined using a Q5000 UV–Vis spectrophotometer (Thmorgan; Quawell, USA) at 260 nm.

Molecular methods

The PCR assay was performed to detect the seven *Eimeria* species in a final volume of 25 µL containing 12.5 µL of 2*PCR Premix (TIANGEN® PCR Master Mix, TIANGEN, China), 20 µM of the forward primers and 20 µM of each reverse primer (Table 1), 1µL of DNA and distilled water with supplement. After an initial denaturing temperature of 95 °C for 10 min, 30 cycles of 98 °C for 30 s, 52.5–65 °C for 30 s, and 72 °C for 1 min were run, followed by a final extension at 72 °C for 1 min with improvement and optimization.

All PCR products were analyzed by separation on 1.5% agarose gel in TAE buffer at 100 V for 30 min, were stained with ethidium bromide, and examined under UV light.

Results

Microscopic examination

Out of the 171 poultry farms screened, 87.72% were positive for *Eimeria* spp. Feixi (96.77%) and Changfeng

Table 1 The primers used in the first PCR assay

Species	Sequence(5′-3′)	PCR product size (bp)	Annealing temperature (°C)
E. brunetti	F[a]-CTGGGGCTGCAGCGACAGGG	183	58
	R[a]-ATCGATGGCCCCATCCCGCAT		
E. mitis	F[a]-GTTTATTTCCTGTCGTCGTCTCGC	330	65
	R[a]-GTATGCAAGAGAGAATCGGGATTCC		
E. praecox	F[a]-CATCGGAATGGCTTTTTGAAAGCG	215	65
	R[a]-GCATGCGCTAACAACTCCCCTT		
E. tenella	F[b]-GTTGCGTAAATAGAGCCCTCT	554	52.5
	R[b]-GTTCCAAGCAGCATGTAACG		
E. maxima	F[b]-GTTGCGTAAATAGAGCCCTCT	152	52.5
	R[b]-ACCAATGCAGAACGCTCCAG		
E. acervulina	F[b]-GTTGCGTAAATAGAGCCCTCT	281	52.5
	R[b]-CAAAAGGTGGCAATGATGCT		
E. necatrix	F[b]-GTTGCGTAAATAGAGCCCTCT	450	52.5
	R[b]-GATCAGTCTCATCATAATTCTCGCG		

[a] Primer previously described (Haug et al. 2007)

[b] Primer previously described (You 2014)

country (93.75%) had the higher infection rates of the collected samples, followed closely by Feidong (89.29%) and Lujiang country (87.10%). The prevalence in Fengtai (82.14%) and Quanjiao (71.43%), were relatively lower (Fig. 1).

The morphological features of the isolated *Eimeria* species revealed that six different species to be present in the examined samples (*E. tenella*, *E. necatrix*, E. *brunette*, *E. mitis*, *E. maxima*, and *E. acervulina*) (Fig. 2).

Molecular identification

All positive samples contained multiple infections, with two to five different species of *Eimeria* involved in the manifestation of the disease in chickens. There were no positive samples that were infected with either a single species or all seven species of *Eimeria*. The data revealed

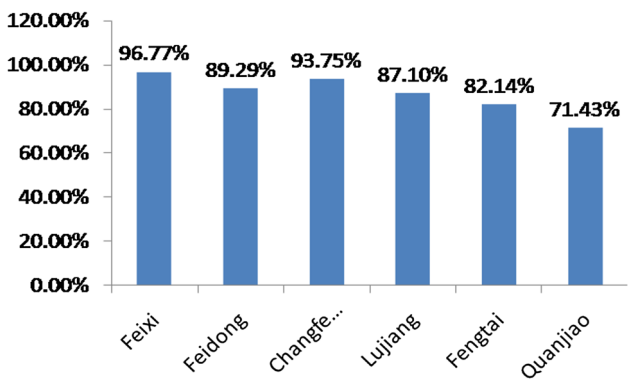

Fig. 1 Prevalence of infection with species of *Eimeria* in chickens in various sites in Anhui province

that *E. tenella*, *E. necatrix*, *E. brunetti*, *E. acervulina*, *E. mitis*, and *E. maxima* existed in the poultry farms of Anhui by molecular examination using a species-specific PCR assay (Fig. 3). However, the prevalence of each species varied. These findings suggest that *E. tenella* is the dominant species (80.67%) in Anhui province, followed by *E. necatrix* (68%), *E. mitis* (55.33%), *E. maxima* (54.67%), *E. brunetti* (44.67%), and *E. acervulina* (2.67%), as presented in Table 2.

The most prevalent combinations are *E. tenella*, *E. maxima*, *E. necatrix*, *E. brunette*, and *E. mitis* (26.67%), followed by *E. tenella*, *E. maxima*, and *E. necatrix* (19.33%). *E. necatrix* was most frequently involved in multiple infections, as shown in Table 3.

Discussion

Chickens are one of the most important food-producing animals. Chicken coccidiosis has emerged and spread rapidly worldwide, thereby posing a significant threat to the poultry industry (Ogedengbe et al. 2011). The aim of the present study was to determine the infection prevalence of *Eimeria* species in chickens from Anhui province, China. Hence, the determination of infection prevalence was investigated in these regions. Additionally, this study was conducted during the four months when coccidiosis has been reported to increase due to increased floor wetness and due to the more humid weather in Anhui province. The increased prevalence of disease may be correlated with the favorable ambient temperature and relatively higher

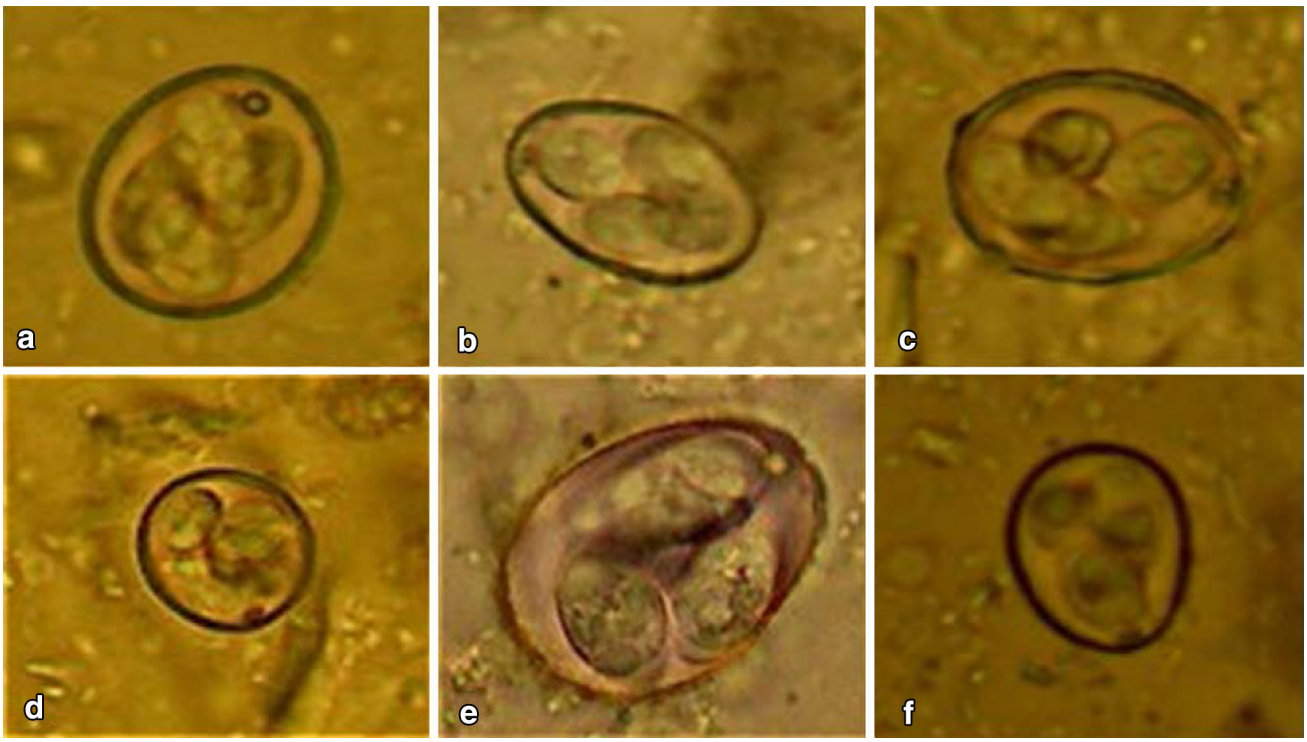

Fig. 2 Oocysts of *Eimeria* spp. identified in fecal samples collected from different poultry farms. **a** *E. tenella*; **b** *E. necatrix*; **c** *E. brunette*; **d** *E. mitis*; **e** *E. Maxima*; **f** *E. acervulina*

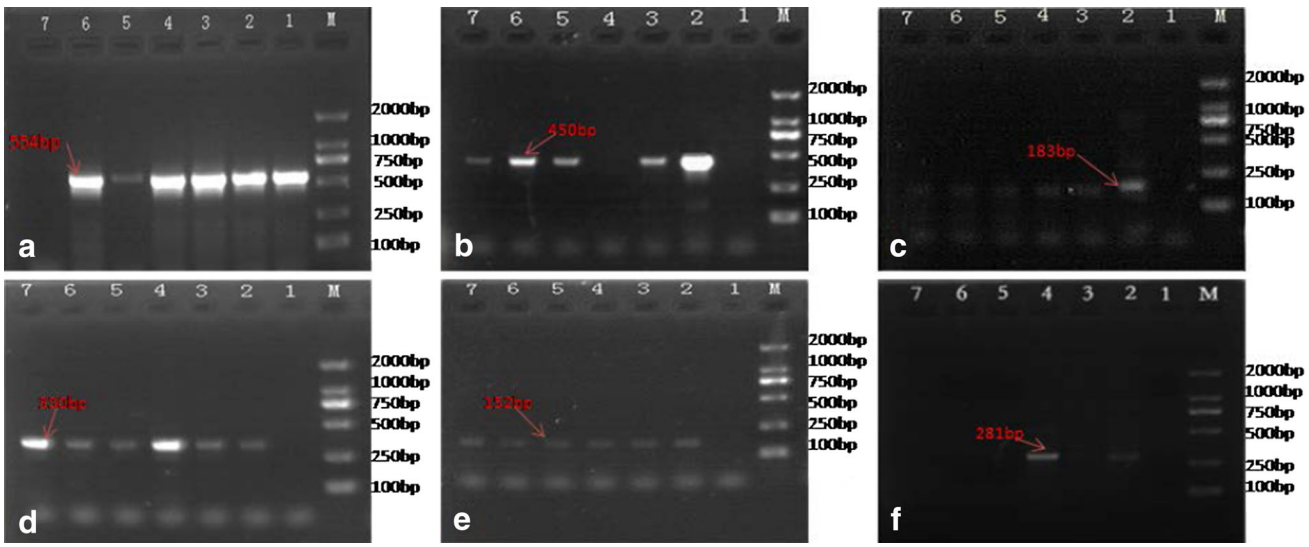

Fig. 3 Results obtained from PCR following 1.5% agarose gel electrophoresis. *Lines* LM D2000 DNA marker. *L1–L7* samples: **a** *E. tenella* 554 bp; **b** *E. necatrix* 450 bp; **c** *E. brunetti* 183 bp; **d** *E. mitis* 330 bp; **e** *E. maxima* 152 bp; and **f** *E. acervulina* 281 bp

humidity by promoting the oocyst sporulation and survivability (Bachaya et al. 2012).

In the present study, six species were detected in the faeces of the chickens that we investigated in Anhui Provence. *E. praecox* is the most frequent but also not found at all. The establishment of different anticoccidial programs could limit the occurrence of some *Eimeria* species more than others, causing changes in the population of these parasites (Carvalho et al. 2011). Additionally, different types animal handling practices can also affect the prevalence of *Eimeria* species. The overall prevalence of coccidian infection obtained in this study (87.72%), was lower than the 96% found in the Southern Region of Brazil (Moraes et al. 2015). However, it was higher than earlier

Table 2 Prevalence of *Eimeria* spp. of chicken using PCR

Regions	*E. tenella* (%)	*E. maxima* (%)	*E. acervulina* (%)	*E. necatrix* (%)	*E. brunetti* (%)	*E. mitis* (%)
Feixi	83.33	66.67	6.67	66.67	63.33	50.00
Feidong	84.00	48.00	4.00	84.00	52.00	52.00
Changfeng	80.00	46.67	3.33	76.67	23.33	46.67
Lujiang	85.19	48.15	0	40.74	33.33	74.07
Fengtai	78.26	60.87	0	73.91	47.83	52.17
Quanjiao	66.67	60.00	0	66.67	53.33	60.00
Total	80.67	54.67	2.67	68.00	44.67	55.33

Table 3 Frequency and prevalence of chickens with a mixed infection in domestic farms

Regions	Te + Ne (%)	Te + Ne + B (%)r	Te + Mx + Ne (%)	Te + Mx + Ne + Br (%)	Te + Mx + Ne + Mi (%)	Te + Ne + Br + Mi (%)	Te + Mx + Ne + Br + Mi (%)
Feixi	3.33	3.33	10.00	30.00	3.33	16.67	33.33
Feidong	4.00	4.00	20.00	8.00	12.00	32.00	16.00
Changfeng	6.67	13.33	33.33	0	16.67	13.33	16.67
Lujiang	3.70	3.70	33.33	7.41	3.70	11.11	18.52
Fengtai	0	8.70	4.35	13.04	21.74	4.35	43.48
Quanjiao	6.67	6.67	6.67	13.33	13.33	6.67	40.00
Total	4.00	6.67	19.33	12.00	11.33	14.67	26.67

Te, *E. tenella*; Ne, *E. necatrix*; Ac, *E. acervulina*; Mi, *E. mitis*; Mx, *E. maxima*; Br, *E. brunetti*

reports from some areas: 81.03% in two north Indian states viz., Uttar Pradesh and Uttarakhand (Kumar et al. 2015), 31.5% in Khuzestan, southwest Iran (Hamidinejat et al. 2010), and 66.8% in Karbala and Babylon province, Iraq (Hamza et al. 2015). The higher prevalence of 87.71% in the current study reveals that the control of coccidiosis has decreased in Anhui province, especially in farms housing a large or medium number of chickens. The prevalence of *Eimeria* infection was the highest in the poultry farms of Feixi (96.77%) compared to the other farms. Perhaps since the poultry industry in Feixi has a long history, chickens with coccidian exhibit or develop resistance to most drugs, thereby rendering the anticoccidials inefficient and leading to clinical failure. Our results seem to agree with the observations made previously by other investigators that suggest the occurrence of mixed infection with more than one species of *Eimeria* is very common, and infection with *E. tenella* and *E. necatrix* are the most prevalent species worldwide (Bhaskaran et al. 2010). We found that domestic chicken farms in Anhui are populated with five species of *Eimeria*: *E. tenella*, *E. maxima*, *E. necatrix*, *E. brunette*, and *E. mitis* (23.39%), followed by *E. tenella*, *E. maxima* and *E. necatrix* (16.96%). Of these, *E. necatrix* exhibited the highest participation in multiple infections (Table 3). Specifically, the epidemic of *E. necatrix* and *E. mitis* is increasing and effort should be made to strengthen preventative measures. *E. acervulina* have the highest reproductive potential (Williams 2001) and in mixed infections, *E. acervulina* reduces the oocyst production of *E. tenella*, *E. maxima*, *E. necatrix*, and *E. brunetti*. As a result, it is difficult to find *E. acervulina* in mixed infections of chicken coccidiosis.

During the past decade, PCR methods based on conserved ITS1 regions of rDNA (Hamidinejat et al. 2010) were developed for the differentiation of chicken coccidian species (Kumar et al. 2014). PCR methods are used for coccidian species determination due to the high specificity and sensitivity of this assay. Traditional methods for species differentiation have proved to be an extremely subjective species identifier. In particular, all strains of both species contain subspherical oocysts, and accurate identification also requires highly trained personnel (Györke et al. 2013) and is limited by the overlap of characteristics among the different species (Haug et al. 2007). Furthermore, multiple infections with *Eimeria* spp. was more frequent than a single infection (Carvalho et al. 2011), thereby making it unreliable to distinguish species based on traditional methods (Aarthi et al. 2010). In our experience, diagnostic multiplex PCR systems used for the primary detection of infectious agents are more difficult to optimize, with less sensitivity and reproducibility. As a result, we chose to work with single-species PCR systems. The PCR technique we used described by Haug et al. and Myung-Jo You was specific for *Eimeria* species with

improvement and optimization. We included primers for each of the seven species (Table 1), of which six species (*E. tenella*, *E. acervulina*, *E. maxima E. necatrix*, *E. brunette*, and *E. mitis*) could be reproducibly detected in this study.

To our knowledge, this is the first report on the prevalence of the chicken *Eimeria* species based on PCR in Anhui province. The present study provided evidence that chicken coccidia is widely prevalent in chicken farms in Anhui province, China. The high prevalence of coccidian infection in chicken farms indicates that it is necessary to carry out suitable control programs. Moreover, careful attention should be paid to highly prevalent coccidian species (i.e., *E. tenella*, *E. necatrix*, and *E. brunetti*) to control the outbreak of coccidiosis in chickens. Integrated strategies should be undertaken to eliminate potential risk factors in chicken farms. In conclusion, coccidiosis is a serious and economically important disease in Anhui province, and is one of the major constraints that hamper chicken production and productivity. The most significance is that vaccine development can focus on the most prevalent strains in an effort to control this disease.

Acknowledgements The authors sincerely acknowledge the assistance and work of members of the Pharmacology and Toxicology Laboratory at Anhui Agricultural University, as well as the regional staff and volunteers for their help in the collection of fecal samples from the areas in Anhui province.

Author contributions YH and MZ designed experiments; YH and XR carried out experiments; YH and LL analyzed experimental results; YH wrote the manuscript.

Compliance with ethical standards

Conflict of interest The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Aarthi S, Raj GD, Raman M, Gomathinayagam S, Kumanan K (2010) Molecular prevalence and preponderance of *Eimeria* spp. among chickens in Tamil Nadu, India. Parasitol Res 107:1013–1017

Al-Natour MQ, Suleiman MM, Abo-Shehada MN (2002) Flock-level prevalence of *Eimeria* species among broiler chicks in northern Jordan. Prev Vet Med 53:305–310

Bachaya HA, Raza MA, Khan MN, Lqbal Z, Abbas RZ, Murtaza S, Badar N (2012) Predominance and detection of different *Eimeria* species causing coccidiosis in layer chickens. J Anim Plant Sci 22:597–600

Bhaskaran MS, Venkatesan LR, Tirunelveli JH, Sriraman R (2010) Sequence diversity of internal transcribed spacer-1(ITS-1) region of *Eimeria* infecting chicken and its relevance in species identification from Indian field samples. Parasitol Res 106:513–521

Carvalho FS, Wenceslau AA, Teixeira M, Matos Carneiro JA, Melo AD, Albuquerque GR (2011) Diagnosis of *Eimeria* species using traditional and molecular methods in field studies. Vet Parasitol 176:95–100

Godwin RM, Morgan JAT (2014) A simple, one-tube assay for the simultaneous detection and diagnosis of ten Australian poultry *Eimeria*. Electrophoresis 35:494–502

Györke A, Pop L, Cozma V (2013) Prevalence and distribution of *Eimeria* species in broiler chicken farms of different capacities. Parasite 20:50

Hamidinejat H, Seifiabad MR, Mayahi M, Borujeni MP (2010) Characterization of *Eimeria* species in commercial broilers by PCR based on ITS1 regions of rDNA. Iran J Parasitol 5:48–54

Hamza DM, Al-Massodi HRH, Jeddoa ZMA (2015) Molecular detection and discrimination of three poultry *Eimeria* species in Karbala and Babylon province, Iraq. Int J Curr Res Rev 7:13–19

Haug A, Thebo P, Mattsson JG (2007) A simplified protocol for molecular identification of *Eimeria* species in field samples. Vet Parasitol 146:35–45

Kumar S, Garg R, Moftah A, Clark EL, Macdonald SE (2014) An optimised protocol for molecular identification of *Eimeria* from chickens. Vet Parasitol 199:24–31

Kumar S, Garg R, Ram H, Maurya PS, Banerjee PS (2015) Gastrointestinal parasitic infections in chickens of upper gangetic plains of India with special reference to poultry coccidiosis. J Parasit Dis 39:22–26

Li W, Han CX, Chang D, Song MX, Lu YX (2009) Investigation of coccidia fauna in chickens in Harbin prefecture of Heilongjiang province. Chin Vet Sci 39:821–824 **(in Chinese)**

Mcdougald LR, Fuller L, Mattiello R (1997) A survey of coccidian on poultry farms in Argentina. Avian Dis 41:923–929

Moraes JC, França M, Sartor AA, Bellato V (2015) Prevalence of *Eimeria* spp. in broilers by multiplex PCR in the southern region of Brazil on two hundred and fifty farms. Avian Dis 59:277–281

Ogedengbe JD, Hanner RH, Barta JR (2011) DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). Int J Parasitol 41:843–850

Shang PP, OuYang WQ, Fu C, Liu W (2011) Reseach of the efficacy of 5% diclazuril nanoemulsion against chicken coccidiosis. J Northwest A&F Univ 39:19–23

Shivaramaiah C, Barta JR, Hernandez-Velasco X (2014) Coccidiosis: recent advancements in the immunobiology of *Eimeria* species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. Vet Med Res Rep 5:23–34

Sun XM, Pang W, Jia T, Yan WC, He G (2009) Prevalence of *Eimeria* species in broilers with subclinical signs from fifty farms. Avian Dis 53:301–305

Suo XCJP (2004) Poultry coccidiosis. China Agricultural Press, Beijing

United States Department of Agriculture (2015) Broiler meat summary selected countries. [Internet]. [cited 2015]. http://apps.fas.usda.gov/psdonline/circulars/livestock_poultry

Vrba V, Poplstein M, Pakandl M (2011) The discovery of the two types of small subunit ribosomal RNA gene in *Eimeria mitis* contests the existence of *E. mivati* as an independent species. Vet Parasitol 183:47–53

Williams RB (2001) Quantification of the crowding effect during infections with the seven *Eimeria* species of the domesticated fowl: its importance for experimental designs and the production of oocyst stocks. Int J Parasitol 31:1056–1069

You M-J (2014) Detection of four important *Eimeria* species by multiplex PCR in a single assay. Parasitol Int 63:527–532

Zaman MA, Iqbal Z, Abbas RZ, Khan MN (2012) Anticoccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. Parasitology 139:237–243