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Prevalence of Coccidial Infection in Domestic Geese in Eastern China

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Abstract: Coccidiosis causes diarrhoea, dehydration and death in geese. Eastern China is a large goose-raising area in China but the coccidial infection status in geese in this region has not been reported as so far. To understand Coccidia species and infection rate, fecal samples were collected from 146 randomly selected clinically healthy domestic goose populations between August 2010 and July 2011. Oocysts were separated by a floatation technique using saturated saline. Coccidia species was identified by examining morphological features of the sporulated oocysts and further verified through animal regression test. The results showed that coccidian oocysts were detected in 87.67% of the goose population. Eight different species of the Eimeriidae family were identified, namely *T. parvula* Koltan (90.63%), *E. hermani* Farr (76.56%), *E. stigmosa* Klimes (48.44%), *E. nocens* Koltan (35.94%), *E. fulva* Farr (15.63%), *E. anseris* Koltan (9.38%), *E. farri* Hanson, Levine and Ivens (4.69%) and *I. anseris* Koltan (4.69%). Among them, the first three species were most prevalent. In addition, 87.50% of the farms had at least two commensal Coccidia species, indicating concurrent infection existed widely in geese. The analysis of coccidial infection with age revealed that the infection mainly occurred in geese older than 30 days and the infection rate increased with ages. In summary, the results suggest that the coccidial infection was common in domestic geese in eastern China and measures for prevention and treatment of coccidiosis were needed for this area.

Key words: Coccidial infection, regression, oocytes, goose, prevalence, Eastern China

INTRODUCTION

Goose coccidiosis, a parasitic protozoiasis is caused by a variety of coccidia in domestic and wild geese. It is distributed worldwide and is especially common in domestic geese in Europe and North America (Hanson *et al*., 1957). Generally, it leads to diarrhoea, dehydration and death in geese. As this disease had been thought to be less harmful all the time, few researches which are mainly about investigation and identification of Coccidia species (Soulsby, 1986) have been conducted to study goose coccidiosis. The disease has two distinct types, namely renal coccidiosis and intestinal coccidiosis. Renal coccidiosis is primarily caused by *E. truncate* and has high morbidity and mortality in infected geese.

Intestinal coccidiosis is caused by many Coccidia species such as *E. anseris*, *E. nocens*, *E. stigmosa*, *E. kotlani*, *T. parvula*, *E. fulva*, *E. hermani*, *E. magnalabia* and *E. striata* (Gajadhar *et al*., 1983a; Levine, 1985). In addition, Coccidia species and infection rate have regional differences in geese (Xie *et al*., 1986; Li and Fu, 1994; Arslan *et al*., 2002). The goose industry in China has been developing rapidly in recent years with the raising practice shifting from scattered, back yard style to large-scale intensive barn raising and the number of geese raised increasing sharply year by year. In 2010, the slaughtered geese in China accounted for 93.5% of those in the world and 99.2% of those in Asia while the number of slaughtered geese in eastern China accounted for 55% of those in China (Wang, 2011). The present intensive feeding provides favorable conditions for the occurrence and spread of goose coccidiosis. Currently, goose coccidiosis occurs occasionally in China and causes economic losses in goose industry. After goose coccidiosis was first reported in Jiangsu province (Fu *et al*., 1986), China, Coccidia species in geese were preliminarily surveyed in Guangdong (Xie *et al*., 1986) and Anhui province (Li and Fu, 1994) based on morphological features of sporulated oocysts.

Due to morphological similarity of sporulated oocysts between some Coccidial species, accurate identification cannot be achieved merely on the basis of morphology and other discrimination methods should also be applied (Gajadhar *et al*., 1983a; Levine, 1985). Prior to the present investigation, there had been no survey on the prevalence of Coccidial infection in geese in eastern China. Therefore, the aim of the present study was to accurately investigate coccidial infection in geese in this

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region and to provide a scientific reference for better control of coccidiosis. Researchers identified prevalent Coccidia species and the infection status in goose farms by observing morphological features of oocysts in feces. Animal regression test was also carried to verify the results.

MATERIALS AND METHODS

Sampling area: The investigation was performed in East China. This area has mild weather, moderate rainfall, clear-cut seasonal changes and an average air temperature of 13-16°C. Its animal husbandry holds an important position in China with the output of livestock and livestock products topping in the country. The amount of slaughtered geese was 430 million (about 55% of that in China) in 2010 and is still increasing year by year.

Sample collection: Fecal samples were collected from 146 randomly selected apparently healthy domestic goose populations in seven main geese rearing administrative regions in eastern China between August 2010 and July 2011. The samples, each of 100-200 g were collected at four surrounding points and one central point of each goose house and stroed at 4°C before examination. All these geese were stocked on the floor or reared in pens on the floor. No anti-coccidiosis drug was used for prevention and treatment of coccidiosis.

Separation and sporulation of oocysts: Coccidian Oocysts Per Gram (OPG) of fecal samples were determined by a modified McMaster Technique. Oocysts were then separated using saturated saline floatation technique as previously described by Li and Fu (1994). Fecal samples were mixed well with water by stirring. After fecal dregs were removed by straining the blend through copper mesh (60 mesh) and nylon sieve (260 mesh) in turn, the filtrate was collected and settled freely. Following removal of the supernatant, the pellet was resuspended in saturated sodium chloride solution to float oocysts. Then, the oocysts were sporulated by incubation in 2.5% (w/t) aqueous potassium dichromate solution at 29°C for 5 days.

Preliminary identification of Coccidia species: The sporulated oocysts were dropped on a glass slide and covered with a cover slip. Their morphological features were observed under a microscope at a magnification of 400x. Twenty oocysts were measured to determine the average size of oocysts and sporocysts. The coccidian oocysts were identified based on their sizes and morphological characteristics as previously described (Hanson *et al*., 1957; Levine, 1985).

Animal regression test: The sporulated oocysts of each fecal sample were orally inoculated into four, 20 days old coccidia free goslings. They were kept in coccidia-free environment disinfected by flame and fed on diets not containing any anti-coccidiosis drug. The water was boiled for 10 min and cooled before drinking. After 4-14 days post inoculation, their feces were collected every 12 h. The oocysts were isolated and sporulated. Coccidia species in each sample were identified according to prepatent period of oocysts and morphological characteristics of the sporulated oocysts.

Statistical analysis: Differences in prevalence between different age groups of geese as well as different regions were evaluated using a χ^2-test in SPSS for Windows (Release 17.0 standard Version, SPSS Inc., Chicago, IL) and a value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Coccidia species in domestic geese in Eastern China: Eight different Coccidia species belonging to three genera of the Eimeriidae family were found in the fecal samples collected. Of the *Eimeria* genus, *E. nocens* Koltan (1933), *E. fulva* Farr (1953), *E. hermani* Farr (1953), *E. anseris* Koltan (1932), *E. farri* Hanson *et al.* (1957) and *E. stigmosa* Klimes (1963) were identified. *I. anseris* Koltan (1932) belonging to the *Isospora* genus and *T. parvula* Koltan (1933) belonging to the *Tyzzeria* genus were also found. Morphological features of these Coccidia species are shown in Fig. 1. The morphological feature and prepatent period of each species obtainted by animal regression test were described.

E. nocens: The shape of sporulated oocysts is ovoid but fattened at the micropylar end, 29-34×19-22.5 μm with a mean of 32.23±2.02×20.01±3.10 μm. The oocyst wall is smooth and consists of two layers, the outer layer is pale yellow and the inner is colorless. The micropyle appears to be in the inner wall and is covered by the outer wall. The sporocysts are broadly ellipsoidal, 12.5-15×10-12.5 μm with a mean of 13.64±1.30×10.23±0.75 μm. The oocyst polar granule and oocyst residuum are absent, part of the oocyst wall forms one or more roundish protuberances just below the micropyle. A small Stieda body is present with sporocyst residuum filling the space between sporozoites. The prepatent period is 228 h.

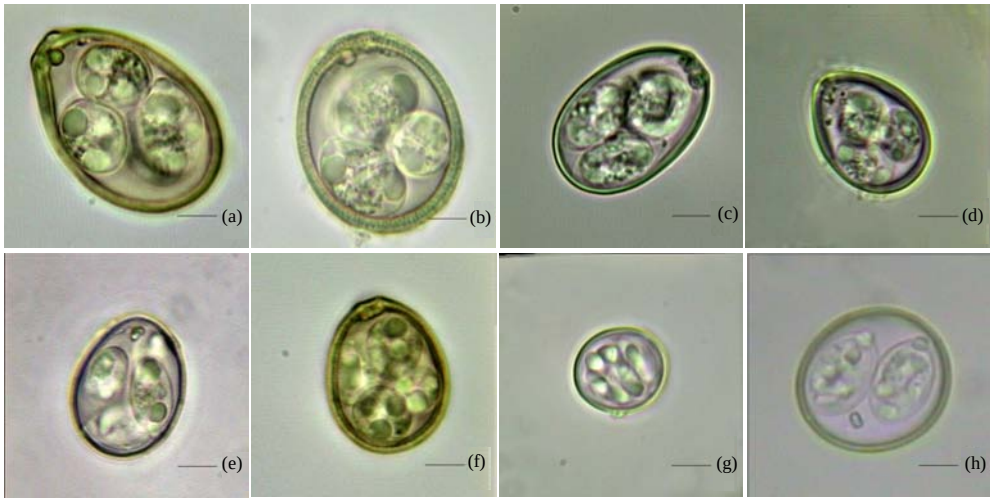

Fig. 1: Sporulated oosysts of eight *Eimeriidae* species; a) *E. nocens* Koltan; b) *E. fulva* Farr; c) *E. hermani* Farr; d) *E. anseris* Koltan; e) *E. farri* Hanson, Levine and Ivens; f) *E. stigmosa* Klimes; g) *T. parvula* Koltan; h) *I. anseris* Koltan. The actual size of each sporulated oosyst was described in the text. Scale bars = 5 μm

E. fulva: The oocysts are broadly ovoid and slightly flattened at the narrow micropylar end, 29.5-33×20-23 μm with a mean of 31.22±1.86×21.7±2.79 μm. The oocyst wall is composed of two layers. The outer layer is brownish yellow, pitted and transversely striated. The inner layer is smooth and colorless. The sporocysts are ovoid, 10-15×8.75-12.5 μm with a mean of 12.48±0.81×10.19±0.93 μm. There is a large polar granule near the micropyle. The oocyst residuum is absent. Sporocyst residuum and a prominent Stieda body are present. The prepatent period is 180 h.

E. hermani: The oocysts are ovoid, fattened and narrowed at the micropylar end, 19-27×10-22 μm with a mean of 22.39±1.56×16.14±1.06 μm. The oocyst wall is composed of two layers which are smooth and colorless. There is a nodular protuberance at each side of the micropyle. The sporocysts are ovoid, 7.5-2.5×5-11.5 μm with a mean of 10.75±1.78×7.81±1.89 μm. The oocyst polar granule and oocyst residuum are absent. Sporocyst residuum and Stieda body are present. The prepatent period is 108 h.

E. anseris: The oocysts are pear-shaped with a micropyle at the truncate end, 18-23×12.5-17.54 μm with a mean of 21.89±1.41×14.58±1.22 μm. The oocyst wall is smooth, colorless, composed of a single laye and incised sharply to form a plate or shelf across the micropyle. The sporocysts are ovoid and almost completely fill the oocyst, 7.5-10×5-6.25 μm with a mean of 9.56±0.93×5.06±0.49 μm. The oocyst polar granule is absent, oocyst residuum is a mass of amorphous material just beneath the micropyle and forms a seal. The sporocyst residuum is granular and stieda body is not obvious. The prepatent period is 156 h.

E. farri: The oocysts are ovoid to ellipsoidal, 20-22×15-17.5 μm with a mean of 20.40±0.79× 16.67±0.93 μm. The oocyst wall is smooth, colorless or pale yellow, composed of a single layer. The micropyle is absent. The sporocysts are long ovoid, 6.5-13×4.5-5.25 μm with a mean of 10.42±0.36×4.98±0.32 μm. There is no oocyst polar granule with oocyst residuum present. A sporocyst residuum and a stieda body are present. The prepatent period is 120 h.

E. stigmosa: The shape of sporulated oocysts is broadly ovoid, a little narrow at the micropyle end, 17-27×10-22 μm with a mean of 21.25±1.26×16.84±1.11 μm. The oocyst wall consists of a single layer which is punctate, radially striated and brown. The sporocysts are ovoid, 11.25-15×7.5-12.5 μm with a mean of 12.50±0.81×10.17±1.00 μm with a sporocyst residuum and a Stieda body are present. There are one or two oocyst polar granules, oocyst residuum is absent. The prepatent period is 108 h.

Isospora anseris: The oocysts are ellipsoidal, 21-23×22-25 μm with a mean of 21.55±1.21×23.61±1.22 μm. The oocyst wall is smooth, colorless, composed of a single layer. The sporocysts are ovoid, 15-17.3× 11.3-13.7 μm with a mean of 15.5×12.5 μm. There are two

sporocysts in a sporulated oocyst which contain four sporozoites in each sporocysts. The oocyst residuum and micropyle are absent. A polar granule, a Stieda body and sporocyst residuum are present. The prepatent period is 120 h.

T. parvula: The oocysts are subspherical to spherical, 6-15×4-13 μm with a mean of 11.55±1.21×9.61±1.22 μm. The oocyst wall is smooth, colorless, composed of a single layer. The micropyle is absent. The sporulated oocysts contain eight banana-shaped sporozoites surrounding the residuum. The prepatent period is 120 h.

Coccidial infection in domestic geese in Eastern China: Coccidial infection, mainly concurrent infection was found in 87.67% (128/146) of the surveyed goose populations, ranged from 82.35-90% in seven locations. However, there were no statistically significant difference in prevalence among seven different regions ($p>0.05$) (Table 1). Two Coccidia species were commensal in 39.06% of the infected goose populations and three Coccidia species were commensal in 28.13% of the infected goose populations. Furthermore, 87.50% of the infected goose populations had more than two commensal Coccidial species (Table 2). In addition, the positive rate of coccidial infection increased with aging in geese. The positive rate of coccidial infection was 12.5% at the age of 1-30 days then rose dramatically at the age of 30-60 days and 60-90 days and reached 100% at the age of older than 90 days. The positive rate at the age of 1-30 days was significantly lower compared to other age groups ($p<0.05$) (Table 3). The differences in OPG were not significant among different age groups ($p>0.05$) (Table 3).

Table 1: Prevalence of coccidial infection in geese in different administrative regions in Eastern China

Geographical locations	Examined No.	Positive No.	Prevalence (%)
Region A	18	16	88.89
Region B	15	13	86.67
Region C	20	18	90.00
Region D	28	25	89.29
Region E	26	23	88.46
Region F	22	19	86.36
Region G	17	14	82.35
Total	146	128	87.67

Table 2: Prevalence of commensal Coccidia species in the 128 infected goose populations

Number of commensal Coccidia species	Positive No.	Prevalence (%)
1	16	12.50
2	50	39.06
3	36	28.13
4	14	10.94
5	8	6.25
6	4	3.13

Infection rates of different Coccidia species in Eastern China: In Eastern China, *T. parvula* Koltan and *E. hermani* Farr were the most prevalent species in the coccidia-positive goose populations followed by *E. stigmosa* Klimes, *E. nocens* Koltan and *E. fulva* Farr. Species of *E. anseris* Koltan, *E. farri* Hanson, Levine and Ivens and *I. anseris* Koltan had lower infection rates in the positive goose populations. The infection rates of these Coccidia species are shown in Table 4.

Goose coccidiosis has drawn less attention compared to bacterial and viral diseases in geese. Here we reported the Coccidia species and infection rates in geese in Eastern China. Fecal samples for the present study were collected from geese of different ages in the rainy season (mainly in June and July) when animals are most susceptible to coccidial infection (Pellerdy, 1974; Levine, 1985). Therefore, the investigation results basically reflect the status of coccidial infection in the region. Differences in Coccidia species and infection rates between seasons were not analyzed due to the limitation of experimental conditions.

In the present investigation, *T. parvula* Koltan, *E. hermani* Farr, *E. stigmosa* Klimes, *E. nocens* Koltan, *E. fulva* Farr, *E. anseris* Koltan, *E. farri* Hanson, Levine and Ivens and *I. anseris* Koltan, all belonging to the Eimeriidae family were found in geese. The former three Coccidia species had higher infection rates. These results are similar to those reported in France and Turkey (Arslan *et al*., 2002). Additionally, the goose Coccidia species isolated in Eastern China were basically the same as those isolated in other parts of China but the number of goose Coccidia species was slightly more than that in southern China (Xie *et al*., 1986) and orther regions (Li and Fu,1994). The observation of oocyst morphology

Table 3: Prevalence and intensity of coccidial infection in the goose populations of different ages

	Number			
Age (days)	Examined	Positive	Prevalence (%)	Average oocyst per gram of faeces of postive samples
1-30	16	2	12.50	5200
30-60	40	38	95.00	4800
60-90	54	52	96.30	5000
90-360	36	36	100.00	4900

Table 4: Coccidia species identified and their prevalence in the 128 infected goose populations

Species	Positive No.	Prevalence (%)
E. nocens Koltan	46	35.94
E. fulva Farr	20	15.63
E. hermani Farr	98	76.56
E. anseris Koltan	12	9.38
E. farri Hanson, Levine and Ivens	6	4.69
E. stigmosa Klimes	62	48.44
I. anseris Koltan	6	4.69
T. parvula Koltan	116	90.63

is an important but not a reliable way for identification of Coccidia species (Levine, 1985) because some sporulated oocysts have very similar features. For more accurate survey researchers performed both morphological examination and animal regression test to distinguish different Coccidia species according to their morphological characteristics and prepatent period. This may interpret why more Coccidia species were isolated in eastern China than other regions of China. Notably, renal coccidiosis caused by *E. truncata* was not found in Eastern China, Southern China and other regions. This Coccidia species mostly lives in wild geese (Gajadhar *et al*., 1983b) and has low infection rate in domestic geese in China.

CONCLUSION

The positive rate of coccidial infection reached 87.67% in the domestic goose populations in eastern China. Most goose populations suffered from mixed infection. Some goose populations showed diarrhea and dehydration occasionally as described by farmers (personal communications). These data indicate that the coccidial infection is prevalent in geese in this region. At present, the majority of field veterinarians and farmers in China do not have enough knowledge about the comprehensive prevention and control of goose coccidiosis. Moreover, no effective monitoring measure is available for this disease. Thus, coccidial infection occurrs frequently, resulting in serious economic loss. In order to effectively control coccidiosis, knowledge about prevention and treatment of coccidiosis should be publicized and a scientific control system should also be established. In summary, the present investigation revealed that the coccidial infection is very common in domestic geese in Eastern China. The study suggested that preventive measures need be taken and effective drugs should be selected for the prevention and treatment of coccidiosis in greese.

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