



PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS

Qingdao (China) - June 15-18, 2016

ISSN 2308-1910

Session Pathology and Hygiene

Gu X., Wang Y., Fang S., Li C., Tao G., Cui P., Suo X., Liu X.

***EIMERIA MEDIA: SELECTION AND CHARACTERIZATION
OF A PRECOCIOUS LINE.***

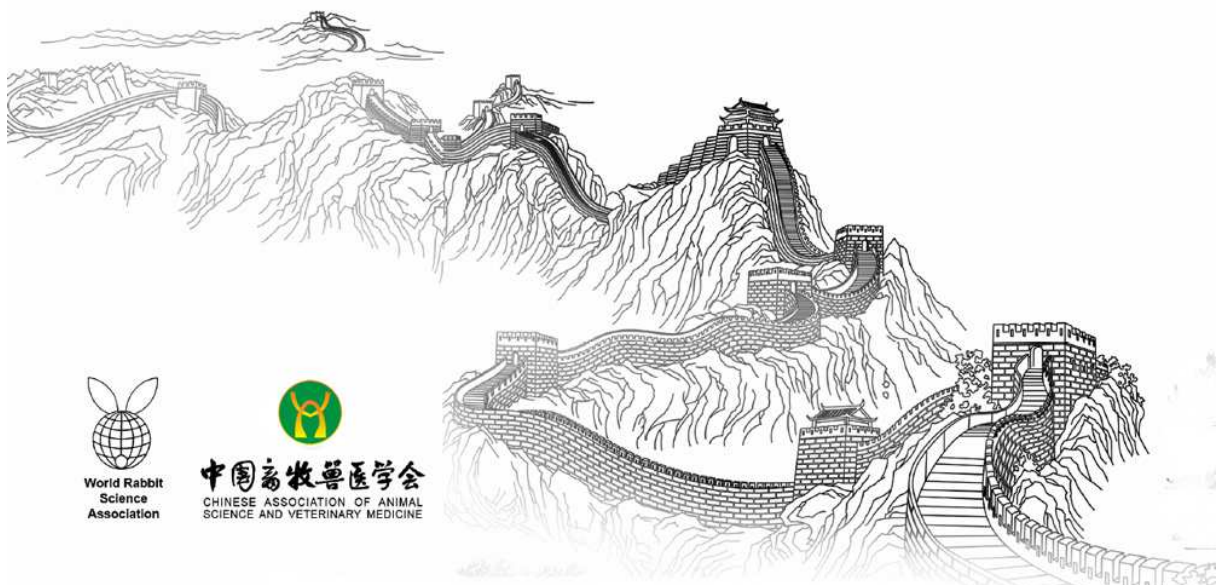
Full text of the communication

+

Poster

How to cite this paper :

Gu X., Wang Y., Fang S., Li C., Tao G., Cui P., Suo X., Liu X., 2016 - Eimeria media: selection and characterization of a precocious line.. Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 531-534 + Poster



***EIMERIA MEDIA*: SELECTION AND CHARACTERIZATION OF A PRECOCIOUS LINE**

Gu X^{1,2}, Wang Y¹, Fang S², Li C¹, Tao G¹, Cui P², Suo X¹, Liu X^{1*}

¹National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, 100193, Beijing, China

²College of Animal Science and Technology, Hebei North University, 075000, Zhangjiakou City, China

*Corresponding author: Xianyong Liu, liuxianyong@cau.edu.cn

ABSTRACT

Coccidiosis, caused by the infection of eimerian parasites, is a common disease of domestic rabbits. As live anticoccidial vaccines have been extensively used for the control of chicken coccidiosis, similar strategy, i.e., formulating the vaccine with attenuated precocious strains of pathogenic eimerian parasites, was expected to be applied for the control of rabbit coccidiosis. In this study, a precocious line of *Eimeria media* was obtained by collecting and successively propagating the first excreted oocysts with 15 generations. Selection for precocious development was accompanied by morphological changes of the sporulated oocysts: each sporocyst contained only one large refractile body in this precocious line; while two smaller refractile bodies could be seen in every sporocyst of the original strain. For the precociously selected line of *E. media*, the prepatent period was reduced from 108 to 70 h. Its fecundity was lowered to 4% of the parental strain. Its pathogenicity was also significantly reduced in term of body weight gain. After inoculation with the precocious line, rabbits were fully protected against homologous challenge with parental strain, though few oocysts detected in the feces after the challenge. These results together indicates this precocious line of *E. media* is a promising component of the anticoccidial vaccine against rabbit coccidiosis.

Key words: Rabbit coccidiosis, *Eimeria media*, Precocious line, fecundity, pathogenicity, immunogenicity

INTRODUCTION

Coccidiosis is an important disease of domestic rabbits. Young rabbits are commonly affected by diarrhea, weight loss and anorexia (Lennox, et al., 2009). *Eimeria media* is widespread in commercial rabbit husbandry (Coudert. 1989). *E. media* is a moderately pathogenic species in domestic rabbit (Jing et al., 2011), though infection with large number of oocysts interferes with digestion and absorption and significantly reduces body weight gain. Inoculation with live attenuated oocysts have been proved to provide sufficient protection against coccidiosis (Akpo et al., 2012). Attenuated strains of 6 *Eimeria* species were obtained by the selection for early development of endogenous stages (Licois, et al., 1990; Licois, et al., 1994; Coudert, et al., 1995; Licois, et al., 1995; Pakandl, 2005; Pakandl, et al., 2006). This study describes the selection of a precocious line of *E. media* strain and the characterization of its morphology, reproduction, pathogenicity and immunogenicity.

MATERIALS AND METHODS

Selection for the precocious line of *E. media*

The original strain of *E. media* (OrEm) was isolated from a fecal sample collected from Hebei province in 2008. Oocysts were recovered from infection with a single oocyst. The precocious line (PrEm) was

obtained by successive selection of the first oocysts shed in the feces by the rabbits inoculated with oocysts of the previous generation. The detail for the selection was summarized in Table 1.

Pathogenicity and immunogenicity test

Pathogenicity of OrEm and PrEm were compared. Seven groups of 3 rabbits were used. Six groups were inoculated with 1×10^2 , 1×10^3 or 1×10^4 sporulated oocysts of PrEm and OrEm, respectively. The remaining groups were used as non-infective control. Total oocyst output was monitored from day 3 to 10 post-inoculation (PI). Body weight of all animals were measured once a week.

For immunogenicity test, 8 groups of 3 rabbits were used. Two groups were unimmunized and unchallenged (UUC) and unimmunized and challenged (UCC), respectively. Six groups were inoculated with 1×10^2 , 1×10^3 or 1×10^4 sporulated oocysts of PrEm and OrEm, respectively. After 14 days PI, rabbits except those in UUC group were challenged with 1×10^4 OrEm oocysts. Body weight was measured once a week for all animals during the assay. Total oocyst output was monitored daily from days 3 and 10 post-inoculation (PI) or days 3 and 10 post-challenge (PC).

Statistical Analysis

Statistical analysis was performed by one-way ANOVA of SPSS software (Version 17.0). Data were expressed as mean \pm standard deviation, and statistical significance was determined using Student's *t*-test; *indicates $p < 0.05$; while ** indicates $p < 0.01$.

RESULTS AND DISCUSSION

1. Development of the precocious line

The prepatent period reduced from 108 to 70 h after 15 successive generations of precociousness selection. The prepatent periods were distinctly decreased during the passages of P2, P6, P13 and P16 (Table 1).

Table 1: Selection of the precocious line of *Eimeria media* (PrEm)

| Strain inoculated | Number of oocysts inoculated (10^4) | Prepatent period (h) | Strain obtained |
|-------------------|---|----------------------|-----------------|
| P0 | Single oocyst | 114 | P1 |
| P1 | 0.5 | 114 | P2 |
| P2 | 0.5 | 96* | P3 |
| P3 | 0.5 | 94 | P4 |
| P4 | 5 | 103 | P5 |
| P5 | 5 | 98 | P6 |
| P6 | 5 | 91* | P7 |
| P7 | 5 | 91 | P8 |
| P8 | 5 | 93 | P9 |
| P9 | 5 | 90 | P10 |
| P10 | 10 | 91 | P11 |
| P11 | 10 | 90 | P12 |
| P12 | 10 | 89 | P13 |
| P13 | 20 | 79* | P14 |
| P14 | 20 | 79 | P15 |
| P15 | 20 | 70* | |

2. The morphological difference of refractile bodies between parental and precocious lines

In OrEm sporulated oocysts, a small refractile body could be observed in each sporozoite (Fig 1). However, in most PrEm sporulated oocysts, one large refractile body could only be found in each

sporocyst. This morphological difference between the parental and precocious strains of *E. media* is similar to the previous study (Pakandl, 2001).

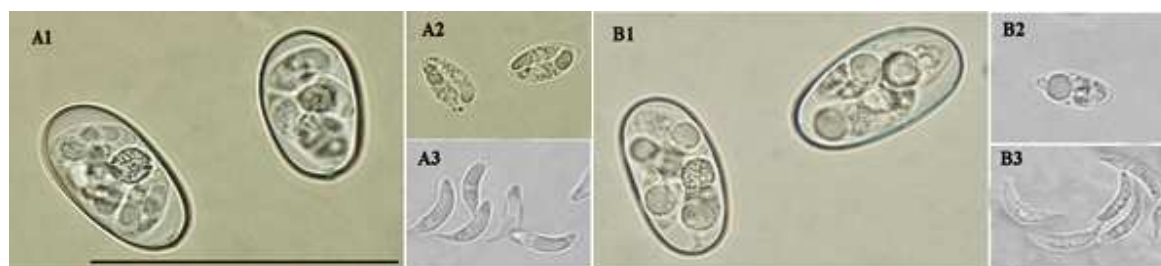


Figure 1: Comparison of refractile bodies between OrEm (A) and PrEm (B). Bar = 50 µm

3. Reproduction, pathogenicity and immunogenicity of the precocious line PrEm

The oocyst production of the precocious strain was 4% of that of the parent strain when rabbit was inoculated with 1×10^2 oocysts of either strain (Fig. 2A and 2B).

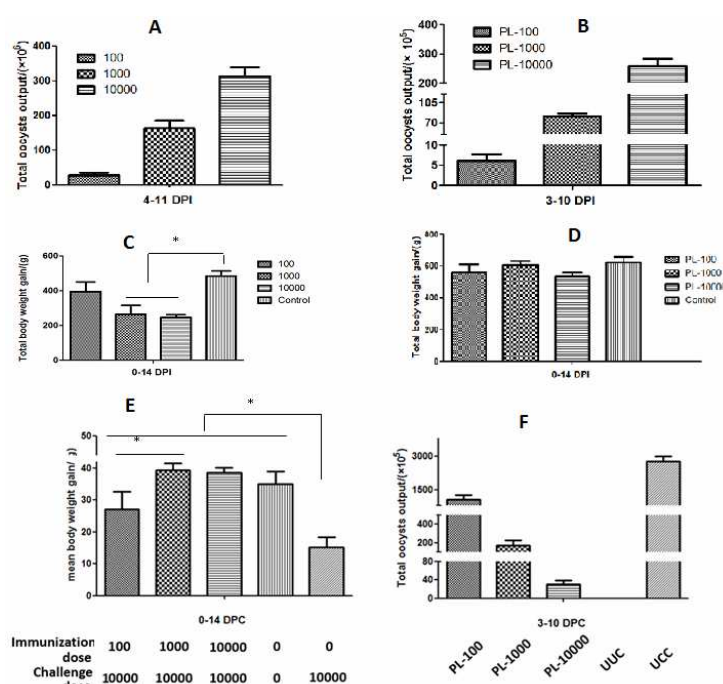


Figure 2 : Reproduction, pathogenicity and immunogenicity of the precocious line PrEm. Oocyst production when rabbits were inoculated with different doses of either OrEm (A) or PrEm (B). Body weight gain of rabbits after inoculation with different doses of wild type OrEm (C) and PrEm (D). Body weight gain (E) and oocyst production (F) of rabbits challenged with 1×10^4 OrEm oocysts.

No death was found in all rabbits during the test. Compared with UUC group, the group inoculated with 1×10^2 OrEm oocysts caused decrease of the weight gain but without significant difference for the weight gain; while significant differences was found in groups with dosages of 1×10^3 ($P < 0.05$) and 1×10^4 ($P < 0.05$), respectively. No difference of body weight gain was observed between UUC group and the rabbits given 1×10^2 , 1×10^3 and 1×10^4 PrEm oocysts (Fig. 2C and 2D), indicating that the pathogenicity of PrEm markedly reduced when compared to that of OrEm.

Immunogenicity: The UCC group had a statistically significantly lower weight gain than that of the UUC group, confirming the pathogenicity of the challenge ($P < 0.05$). Furthermore, there were no statistically significant difference between the weight gain of each VC group and that of UUC group, and each VC group had a statistically significant higher weight gain than that of the UCC group ($P < 0.05$), confirming that the vaccinated rabbits were immune to the virulent challenge of the parent strain.

There was no significant difference of the weight gain between one of the vaccinated and challenged groups and the UUC group. Rabbits in each vaccinated and challenged group had a significant higher weight gain than that of the UCC group ($P < 0.05$). These data confirmed that the vaccinated rabbits, either with parental strain or precocious strain, developed protective immunity against the challenge with the virulent parent strain. It also showed that rabbits had similar body weight gain after immunization with 1×10^3 or 1×10^4 PrEm oocysts compared to that of the UUC group, while rabbits immunized with 1×10^2 PrEm oocysts had significantly reduced body weight gain, suggests that full protection was developed after an immunization with 1×10^3 or 1×10^4 PrEm oocysts.

CONCLUSIONS

Attributes of the precocious line of *E. media*, described here, suggest that it is a suitable candidate for inclusion in a live attenuated vaccine for coccidiosis in rabbits.

ACKNOWLEDGEMENTS

This research was supported by China Agricultural Research System (CARS-44) and the National Natural Science Foundation of China (Key project, No. 31330076).

REFERENCES

- Lennox, A. M. and Kelleher, S. 2009. Bacterial and parasitic diseases of rabbits. *Vet. Clin. North Am. Exot. Anim. Pract.* 12: 519–530.
- Coudert P. 1989. Some peculiarities of rabbit coccidiosis. *Proceedings of the 5th International Coccidiosis Conference on Coccidia and Intestinal Coccidiomorphs*, 17-20, Tours, France, pp 481-488
- Fa Jing, Guangwen Yin, Xianyong Liu, Xun Suoa, Yinghe Qin. 2011. Large-scale survey of the prevalence of Eimeria infections in domestic rabbits. *Parasitol Res.* 2012, 110(4):1495-1500
- Akpo Y, Kpodékon MT, Djago Y, Licois D, Youssao IA. 2012. Vaccination of rabbits against coccidiosis using precocious lines of Eimeria magna and Eimeria media in Benin. *Vet Parasitol.* 2012, 184(1):73-76.
- Licois D, Coudert P, Boivin M, Drouet-Viard F, Provôt F. 1990. Selection and characterization of a precocious line of Eimeria intestinalis, an intestinal rabbit coccidium. *Parasitol Res.* 1990;76(3):192-8.
- Licois D, Coudert P, Drouet-Viard F, Boivin M. 1994. Eimeria media: selection and characterization of a precocious line. *Parasitol Res.* 80(1):48-52.
- Coudert P, Licois D, Drouet-Viard F. 1995: Eimeria species and strains of the rabbits. In: J. Eckert, R. Braun, M.W. Shirley and P. Coudert (Eds.), Guidelines on techniques in coccidiosis research. *European Commission, Directorate-General XII, Science, Research and Development Environment Research Programme*, pp. 52–73.
- Licois D, Coudert P, Drouet-Viard F, Boivin M. 1995. Eimeria magna: pathogenicity, immunogenicity and selection of a precocious line. *Vet Parasitol.* 60(1-2):27-35.
- Pakandl M. 2005. Selection of a precocious line of the rabbit coccidium Eimeria flavescens Marotel and Guilhon (1941) and characterisation of its endogenous cycle. *Parasitol Res.* 97(2):150-155.
- Pakandl M, Jelínková A. 2006. The rabbit coccidium Eimeria piriformis: Selection of a precocious line and life-cycle study. *Vet Parasitol.* 137(3-4):351-354
- Pakandl M, Licois D, Coudert P, 2001. Electron microscopic study on sporocysts and sporozoites of parental strains and precocious lines of rabbit coccidia Eimeria intestinalis, E. media and E. Magna. *Parasitol Res.* 87:63-66

***EIMERIA MEDIA*: SELECTION AND CHARACTERIZATION OF A PRECOCIOUS LINE**

GU X^{1,2}, WANG Y¹, FANG S², LI C¹, TAO G¹,
CUI P², SUO X¹, LIU X^{1*}



1 National Animal Protozoa Laboratory & College of Veterinary Medicine, 2 College of Animal Science and Technology, Hebei North University, 075000, Zhangjiakou City, China

* Corresponding author: suoxun@cau.edu.cn

INTRODUCTION

Coccidiosis is an important disease of domestic rabbits. Young rabbits are commonly affected by diarrhea, weight loss and anorexia (Lennox, et al., 2009). *Eimeria media* is widespread in commercial rabbit husbandry (Coudert, 1989). *E. media* is a moderately pathogenic species in domestic rabbit (Jing et al., 2011), though infection with large number of oocysts interferes with digestion and absorption and significantly reduces body weight gain. Inoculation with live attenuated oocysts have been proved to provide sufficient protection against coccidiosis (Akpo et al., 2012). Attenuated strains of 6 *Eimeria* species were obtained by the selection for early development of endogenous stages (Licois, et al., 1990; Licois, et al., 1994; Coudert, et al., 1995; Licois, et al., 1995; Pakandl, 2005; Pakandl, et al., 2006). This study describes the selection of a precocious line of *E. media* strain and the characterization of its morphology, reproduction, pathogenicity and immunogenicity.

Selection for the precocious line of *E. media*

The original strain of *E. media* (OrEm) was isolated from a fecal sample collected from Hebei province in 2008. Oocysts were recovered from infection with a single oocyst. The precocious line (PrEm) was obtained by successive selection of the first oocysts shed in the feces by the rabbits inoculated with oocysts of the previous generation. The detail for the selection was summarized in Table 1.

Pathogenicity and immunogenicity test

Pathogenicity of OrEm and PrEm were compared. Seven groups of 3 rabbits were used. Six groups were inoculated with 1×10^2 , 1×10^3 or 1×10^4 sporulated oocysts of PrEm and OrEm, respectively. The remaining groups was used as non-infective control. Total oocyst output was monitored from day 3 to 10 post-inoculation (PI). Body weight of all animals were measured once a week.

For immunogenicity test, 8 groups of 3 rabbits were used. Two groups were unimmunized and unchallenged (UUC) and unimmunized and challenged (UCC), respectively. Six groups were inoculated with 1×10^2 , 1×10^3 or 1×10^4 sporulated oocysts of PrEm and OrEm, respectively. After 14 days PI, rabbits except those in UUC group were challenged with 1×10^4 OrEm oocysts. Body weight was measured once a week for all animals during the assay. Total oocyst output was monitored daily from days 3 and 10 PI or days 3 and 10 post-challenge (PC).

Statistical Analysis

Statistical analysis was performed by one-way ANOVA of SPSS software (Version 17.0). Data were expressed as mean \pm standard deviation, and statistical significance was determined using Student's t-test; * indicates $p < 0.05$; while ** indicates $p < 0.01$.

RESULTS AND DISCUSSION

Development of the precocious line

The prepatent period reduced from 108 to 70 h after 15 successive generations of precociousness selection. The prepatent periods were distinctly decreased during the passages of P2, P6, P13 and P16 (Table 1).

Table 1: Selection of the precocious line of *Eimeria media* (PrEm)

| Strain inoculated | Number of oocyst inoculated ($\times 10^4$) | Prepatent period (h) | Strain obtained |
|-------------------|---|----------------------|-----------------|
| P0 | Single oocyst | 114 | P1 |
| P1 | 0.5 | 114 | P2 |
| P2 | 0.5 | 96* | P3 |
| P3 | 0.5 | 94 | P4 |
| P4 | 5 | 103 | P5 |
| P5 | 5 | 98 | P6 |
| P6 | 5 | 91* | P7 |
| P7 | 5 | 91 | P8 |
| P8 | 5 | 93 | P9 |
| P9 | 5 | 90 | P10 |
| P10 | 10 | 91 | P11 |
| P11 | 10 | 90 | P12 |
| P12 | 10 | 89 | P13 |
| P13 | 20 | 79* | P14 |
| P14 | 20 | 79 | P15 |
| P15 | 20 | 70* | |

The morphological difference of refractile bodies between parental and precocious lines

In OrEm sporulated oocysts, a small refractile body could be observed in each sporozoite (Fig 1). However, in most PrEm sporulated oocysts, one large refractile body could only be found in each sporocyst. This morphological difference between the parental and precocious strains of *E. media* is similar to the previous study (Pakandl, 2001).

Reproduction, pathogenicity and immunogenicity of the precocious line PrEm

The oocyst production of the precocious strain was 4% of that of the parent strain when rabbit was inoculated with 1×10^2 oocysts of either strain (Fig. 2A and 2B). No death was found in all rabbits during the test. Compared with UUC group, the group inoculated with 1×10^2 OrEm oocysts caused decrease of the weight gain but without significant difference for the weight gain; while significant differences was found in groups with dosages of 1×10^3 ($P < 0.05$) and 1×10^4 ($P < 0.05$), respectively. No difference of body weight gain was observed between UUC group and the rabbits given 1×10^2 , 1×10^3 and 1×10^4 PrEm oocysts (Fig. 2C and 2D), indicating that the pathogenicity of PrEm markedly reduced when compared to that of OrEm.

CONCLUSION

Attributes of the precocious line of *E. media*, described here, suggest that it is a suitable candidate for inclusion in a live attenuated vaccine for coccidiosis in rabbits.

MATERIALS AND METHODS

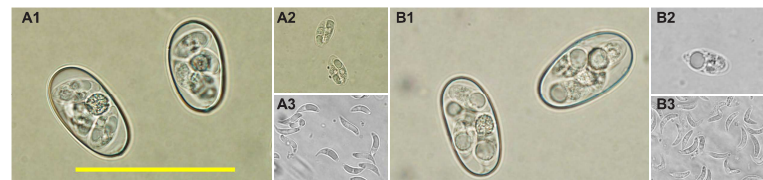


Figure 1: Comparison of refractile bodies between OrEm (A) and PrEm (B). Bar = 50 μ m.

Immunogenicity: The UCC group had a statistically significantly lower weight gain than that of the UUC group, confirming the pathogenicity of the challenge ($P < 0.05$). Furthermore, there were no statistically significant difference between the weight gain of each VC group and that of UUC group, and each VC group had a statistically significant higher weight gain than that of the UCC group ($P < 0.05$), confirming that the vaccinated rabbits were immune to the virulent challenge of the parent strain.

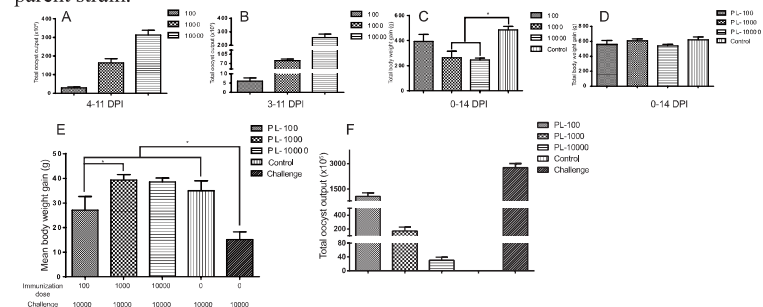


Figure 2: Reproduction, pathogenicity and immunogenicity of the precocious line PrEm. Oocyst production when rabbits were inoculated with different doses of either OrEm (A) or PrEm (B). Body weight gain of rabbits after inoculation with different doses of wild type OrEm (C) and PrEm (D). Body weight gain (E) and oocyst production (F) of rabbits challenged with 1×10^4 OrEm oocysts.

There was no significant difference of the weight gain between one of the vaccinated and challenged groups and the UUC group. Rabbits in each vaccinated and challenged group had a significant higher weight gain than that of the UCC group ($P < 0.05$). These data confirmed that the vaccinated rabbits, either with parental strain or precocious strain, developed protective immunity against the challenge with the virulent parent strain. It also showed that rabbits had similar body weight gain after immunization with 1×10^3 or 1×10^4 PrEm oocysts compared to that of the UUC group, while rabbits immunized with 1×10^2 PrEm oocysts had significantly reduced body weight gain, suggests that full protection was developed after an immunization with 1×10^3 or 1×10^4 PrEm oocysts.

ACKNOWLEDGEMENT

This research was supported by China Agricultural Research System (CARS-44) and the National Natural Science Foundation of China (Key project, No. 31330076).