THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

In Vitro Cultivation of Gametocytes of *Eimeria Tenella* Isolated from Broilers in Jos, Plateau, Nigeria

Kaze Paul Davou
Lecturer, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Jos, Jos, Nigeria
Idris Lawal
Professor, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
Joseph Ajanusi
Professor, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
Saidu Lawal
Professor, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
Adrian Smith
Professor, Department of Zoology, University of Oxford, United Kingdom
Dr. Stephen Preston
Professor, Department of Zoology, University of Oxford, United Kingdom

Abstract:
Sporozoites from a characterized Eimeria tenella sporulated oocyst were used for the study. Nine day old embryonic eggs of chicken had their chario-allantric membrane (CAM) inoculated with 0.1ml (10^4) sporozorites along with penicillin (2000IU) and streptomycin (0.05mg) and incubated at 37^{o}C and 70% humidity. Gametocytes were harvested from the cultures on day 5^{th} – 7^{th} post inoculation from the CAM of dead embryos and concentrarted by centrifigution and washed twice with and stored in phosphate buffer saline (PBS) at 4oC.The gametocytes images from the CAM of the embryonic eggs have similar characteristic structure with that of the ceacum of the broilers.

1. Introduction

Eimeria tenella have severe developmental stages in their life cycles in chickens. They are found in the ceacum and results in heamorrhage responsible for enormous economic losses to the global poultry industry. The understanding of the developmental stages of the parasite through in vitro can be practically appreciated. The life cycle of *Eimeria tenella* is completed within a single host and includes discrete, expansive phases of a sexual reproduction followed by a sexual phase (gametocytes) that results in the production and elimination of large members of oocysts in feaces. (Fernando, 1990). Few *Eimeria* species are capable of developing or forming gemetocytes or completing their life cycles in primary cells in vitro (Doran, 1970). However, *Eimeria tenella* remains the most prevalent and pathogenic of all Eimeria species in chickens (Han et al. 2010). In vitro cultivation of *E. tenella* has increase in recent years supplementing in vivo experimental studies. Long (1965) attempted the cultivation of *E. tenella* in embryos where sponzoites of the parasite adopted successfully in the chorio-allantric membrame of the chicken embryos; thus cells and chicken embryos are used in the culturing of *E. tenella* in vitro. Gametocytes cultivated in chicken embryos provide protection against coccidiosis (Hafeer *et al.,* 2006, 2007; Aktar *et al.,* 2006). The present study is aimed at investigating the development of Eimeria tenella gametocytes in embryonated eggs in the Chario-allantoic membrane (CAM) from broilers in vitro.

2. Methodology

2.1. Ex-sporocystation

Sporulated oocysts of *E. tenella* (local isolates) maintained in the Protozoology laboratory, Parasitology Division, National Veterinary Reseach Institute (N.V.R.I) vom,Plateau State, Nigeria were processed for excystation followed by exsporocystation to release sporozoites (Speer *et al*., 1973). Briefly purified sporulated oocysts were treated with 2.5% sodium hypochlorite for 20 minutes followed by continuous stirring in a vessel containing sterilized glass beads (425-600µm diameter) for 25 minutes on a magnetic stirrer. The excysted material was centrifuged (310×g for 10 minutes), the supernatant was discarded to removed sodium hypochlorite and sediment was given three times washings with phosphate-buffered saline (PBS). The washed sporocyst were suspended in excystation fluid (0.25g trypsin, 4.0g taurodeoxycholic acid and 0.094g magnesium chloride brought to 100ml volume with HBSS at

pH 7.8-8.0) followed by homogenization for 1× 7minutes (cycled on/off for 30 minutes on ice) at 40°C and 5% Co2. The excysted sporozoites were obtained by centrifugation (310× g for 10 minutes) and their concentration were adjusted to 1:8 x 10^3 -2x10^3 per 0.1ml in PBS and stored at 4°C for further use.

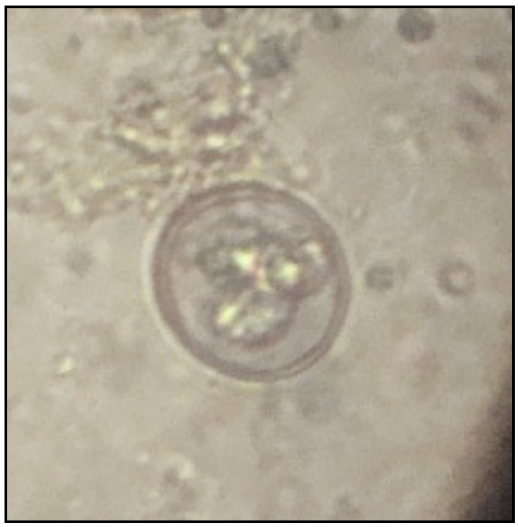

Figure 1: Sporulated oocyst of Eimeria tenella

2.2. Harvesting of Gametocytes

Chicken embryonic eggs (9 days old) were procured from the National Veterinary Reseach Institute hatchery,Poultry Farm, Vom kept and maintained at 39°C and 70% humidity in an incubator. Candling was performed to confirm the viability of the embryos at 12 days of age, 0.lml suspension of sporozoites were inoculated with chicken embryos through chorio-allantoic membrane along with penicillin (2000 IU) and streptomycin (0.05 mg). The embryoes were maintained at 70% humidity for 5-.7 days (Akhtar *et al* 2002). On day 5^{th} -7^{th} post-inoculaton, chorio-allantoic fluid were collected from dead embryos to harvest the gametocytes (Hafeez, 2005). Gametocytes were concentrated by centrifugation (14500g for 5mins), wash twice with sterile PBS and used or stored in PBS at 4°C.

2.3. Microscopy

Images of the *Eimeria tenella* gametocytes were taken using the zeiss Axiorest 100 microscope combrine with the cacl zeiss sony cuber shot 12.1 mega pixels monochrome CN50CC camera of day 5^{th} – 7^{th} post inoculation in CAM embronated eggs.

3. Results

Sporozoites were observed in the chario-allantoic membrane post inoculation and not in the embryo itself. Sporozoites could be seen on the CAM at 2 h p.i. , after which they invaded the CAM cells.The *in vitro* production of gametocytes of *Eimeria species* are still a challenge in coccidiosis research (Hong *et al*., 2013). Gametocytes cultivation *in vitro* had only been observed in some *Eimeria species*. Micro and macrogametes were not observed as there was no cell differentiations. Gametocytes were seen as tiny spherical structures under the microscope as captured in the Micrograph below.

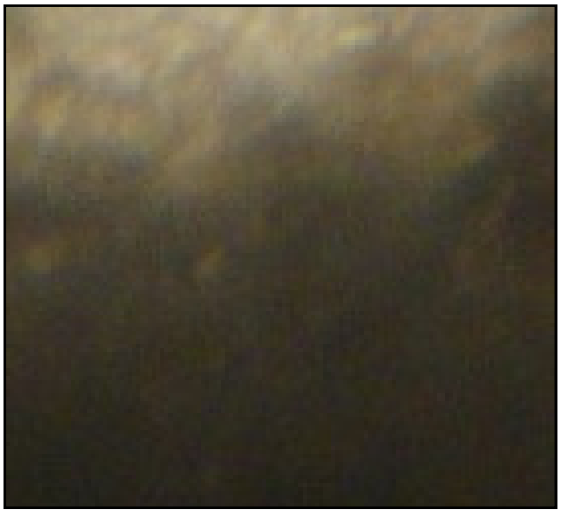

Figure 2: Gametocytes of Eimeria tenella

The embyonated eggs through the CAM provides the basis for gametocytes development and its subsequent production of oocyts, thereby enhances the continuous cultivation and harvesting of the different developmental stages of Eimeria tenella. This indicates that the CAM structures of the embryonic eggs are suitable and same as the dwelling location or cecum of the broilers.

4. Discussion

The sporozoites are the basic structures for development of all the stages of *Eimeria* species because they are only found in the CAM which is suitable for further stages multiplication and developments i.e both asexual and sexual phases and agrees with Woltgang *et al,* 1999 and Jiang *et al*., 2012 reports.The gametocytes cultured in the embryonic eggs-CAM are very similar to those of the broilers as observed by Doran, 1970 and Jiang *et al.,* (2012). Gametocytes development could be shown on the embryonated – CAM structures; indicating that CAM cells have same properties to the ceacum of the broiler and is consistent with the findings of Jiang *et ai*. (2012). There was no differentiation of the gametocytes into micro and macrogametes observed in the CAM of the embryo, thus no fertilization occurred. This may be due to non development of the macro and microgamonts to give rise to the macro and microgametes, suggesting also the non complementary of the embryonated eggs CAM structures characteristics at gametogony to that of the broilers. It may be due to delay in the development of the microgametes as against the past development of macrogametes due to the interference of the embryonic serum, this may also agrees with the above reason. Oocyst formation was not be possible due to the absence of the gametes. This is inconsistent to the findings of Ferguson (2002) stating that fertilization is not obligatory in *Toxplasmoa gondii* development and other coccidian species.This is also dissimilar to report of Jiang *et al*, 2012 that observed oocysts formation thereby indication variation of gametocytes development in the broilers and the CAM structures of the embryonic eggs.

5. References

i. Aktar M., Ayaz M.M., Hayat, C.S., Ashfaque, M., and Hussani, I.(2002). Adaptation of Eimeria tenella sporozoites (local isolates) in embryonated hen's eggs. Pakistan vet. J. 22:40-41

ii. Akhtar, M., Hafeez, M.A., Javed, M.T. and Hussain, I. (2006). Immunogenic Characterization of egg-adapted gametocytes of Eimeria tenella. Parasitology Research, 2007, 100, 1139 – 1141.

iii. Doran, D.J. (1970). "Eimeria tenella form sporozoites to oocysts in cell culture" Proceedings of the Helminthological Society of Washington, Vol. 70, pp 84–92.

iv. Farnando, A.M. (1990). Eimeria: inflections of the intestine in: Coccidiosis of Man and domestic animals Long, P.L. (ed), CRC Press, Boca Raton, pp 63 – 75.

v. Ferguson, D.J.P (2002). Toxoplasma gondii and sex: essential or optional extra. Trends in Parasitology, Vol. 18, No.8, pp 355 -359.

vi. Hafeez, M.A. (2005). Immunogenic characterization of Eimeria tenella gametocytes antigen as vaccine against cocidioses in poultry. Ph.D Thesis, Univ. Agric. Faisalabad, Pakistan.

vii. Hafeez, M.A. Akhtar, M. and Hussain. I. (2006). Protective effects of egg propagated Eimeria tenella (local isolate) gametocytes as Vaccine(s) against mix species of cocidia in chickens. Parasitology Research, 2006, 98, 539 – 544

viii. Hafeez, M.A., Akhtar M. and Ayaz M.M. (2006). Some observations on the adaptation of Eimeria tenella (local isolate) sporozoites on chicken embryos through chorio-allantoic membrane. Journal of Veterinary Science, 2006, 7, 59 -61

ix. Hafeez, M.A., Akhtar, M., Javed, M.T. and Ahsan, H. (2007) Maternal immunization of egg propagated gametocytes vaccine to control Eimeria tenella infections in newly hatch chicks. Parasitology Research, 2007, 100, 1139 – 1141.

x. Han, H.Y., Lin J.J., Zhao, Q.P., Dong, H., Jiang, L.L., Xu, M.Q., Zhu, S.H. and Huang, B. (2010). Identification of differentially expressed genes in early stages of Eimeria tenella by suppression subtractive hybridization and cDNA Microarray. Journal of Parasitology, 2010, Vol. 96, pp 95-102.

xi. Hong, C. Stafanie, W, Sacha, H., Rolf, E. and Micheal Hurth (2013). Development of Eimeria nieschulzi (coccidia, Apicomplexa) Gamonts and oocysts in primary fetal Rat cills. Journal of Parasitology research volume 2013 (2013). Article ID 591520. Long, P.L. (1965). Development of Eimeria tenella in avian embryos. Nature, 1965, 208, 509 – 510.

xii. Jiang. L., Zhao, O., Zhu, S., Han, H. and Doig, H. (2012). Establishment of Eimeria tenella (local isolate) in chicken embryos. Parasite (impact factor: 0.82) Shanghai Veterinary Research 19(3): 285 – 9 Doi:10.1051/parasite.

xiii. Kurth, M. and Entzeroth, R. (2009). "Reporter gene expression in cell culture stages and oocysts of Eimeria niesculzi (coccidia, Apicomplexa). Parasitology Research, Vol.104, no. 2pp 303 -310.

xiv. Speer, C.A., Hammond, D.M., Mahrt, J.L and Roberts, W.L (1973). Structure of the oocysts and sporocysts walls and excystation of sporocysts walls and excystation of sporozoites of Isospora canis. J. parasitol. 59:35-40.

xv. Woltgang, R., Uphoff, M., Hofmann, J., Kerler, G., Seack, K. and Horning, S. (1999). Complete life cycle of Eimeria tenella in vitro. Tokai Exp. Clin. Med. J. Vol. 23, No 6, pp 343.