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实验研究

微小隐孢子虫感染犬肾细胞模型的建立及生长发育过程的研究

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摘要
目的 建立微小隐孢子虫体外感染犬肾细胞（MDCK细胞）模型，并观察其生长发育过程。 方法 利用MDCK细胞为隐孢子虫感染对象，优化隐孢子虫感染MDCK细胞的培养条件，观察隐孢子虫在MDCK细胞中的生长发育过程。将体外感染48 h的细胞培养上清接种小鼠，观察其感染情况。 结果 在含有5%胎牛血清的DMEM培养基中，用1×10^{5}隐孢子虫卵囊感染2.0×10^{5}个MDCK细胞，培养12 h为最佳培养条件。在感染后72 h内，隐孢子虫出现连续发育阶段，包括脱囊、子孢子、裂殖子、裂殖体、滋养体、配子体、合子、薄壁卵囊和厚壁卵囊，在60～72 h内形成卵囊；用感染48 h的细胞培养上清接种于免疫抑制小鼠，10 d后有隐孢子虫卵囊排出。 结论 建立了能稳定用于微小隐孢子虫体外感染的MDCK细胞模型，观察到隐孢子虫的生长发育全过程。

关键词 微小隐孢子虫；感染模型；MDCK细胞；培养

分类号

In vitro Cultivation Model of *Cryptosporidium parvum* in MDCK Cells and its Development

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Abstract

Objective To develop an *in vitro* culture system for *Cryptosporidium parvum* in Madin-Darby canine kidney （MDCK） cell and observe its life cycle （from desquamate to oocyst）. Methods Oocysts of *C. parvum* were co-cultured with MDCK cells *in vitro*. Culture condition was optimized and the life cycle of

C. parvum investigated. Results The optimal culture conditions for *C. parvum* in MDCK cells were 2.0×10^5 cells cultured for 12 h, and infected by 1.0×10^5 oocysts in the Dulbecco′s Modified Eagle Medium with 5% FBS. Following 72 h co-culture, desquamate, sporozoites, trophozoites, meronts, microgametocytes, macrogametocytes, zygote, thin-wall oocyst, and thick-wall oocyst appeared orderly. Between the 60th and 72th hour, many oocysts emerged. Inoculated by the *C. parvum*-infected cell culture supernatant at the 48th hour, the immunosuppressed mice became infected. Conclusion The culture system provides a model for propagation of the parasites and demonstrates a complete *in vitro* life cycle of *C. parvum*.

Key words *Cryptosporidium parvum*; Infection model; MDCK cell; Culture

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