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论著

犬贾第虫携病毒株体外纯培养的建立

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摘要

目的 培养一携带犬贾第虫病毒的犬贾第虫（*Giardia canis*）细胞株。 方法 用蔗糖密度梯度离心-G1耐酸漏斗过滤法纯化犬贾第虫包囊，经口接种5日龄长爪沙鼠（*Meriones unguiculata*），8 d后于其十二指肠无菌收集犬贾第虫滋养体，置改良的 TYI-S-33 培养基中培养，待滋养体在培养管壁上形成细胞单层后进行传代。同时进行冻存和复苏实验，以及纯度、稳定性、细胞生物学特性、微生物污染等4项指标检测。滋养体经液氮冻融3次后3 000 × g离心15 min，取上清，用磷钨酸负染，透射电镜观察病毒粒子。 结果 犬贾第虫滋养体接种14 d 后虫体逐渐适应了培养环境，在培养管壁上形成细胞单层，经上述4项指标检测，证明形成了稳定的犬贾第虫细胞株。电镜观察，见滋养体内有外观球形呈20面体结构、直径约为36 nm的病毒样粒子。 结论 建立了携带GCV的犬贾第虫细胞株的体外纯培养。

关键词 犬贾第虫病毒 体外培养 改良TYI-S-33培养基

分类号

Establishment of In vitro Cultivation of *Giardia canis* Trophozoites Infected with *Giardia canis* Virus

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Abstract

Objective To cultivate a *Giardia canis* isolate with *G.canis* virus（GCV）. Methods Five-day-old *Meriones unguiculatus* was infected with the cysts of G. canis isolated from dogs in Changchun and purified by sucrose density gradient centrifugation-G1 acid funnel filtration method. Trophozoites were isolated aseptically from the duodenum of the infected rodent after 8 days，then transferred to modified TYI-S-33 medium and cultivated at 37 ℃. The trophozoites were centrifuged with 3 000 × g，15 min after liquid nitrogen freeze-thawing three times and the supernatant stained negatively by phosphotungstic acid was observed with transmission electron microscope. Results *G. canis* trophozoites which adapted gradually to the environment and grew a cellular monolayer after 14 days were examined by freezing and thawing experiment，purity quotient，stability，biology characteristics and microbial contamination detection. The results demonstrated that a stable *G.canis* trophozoite cell isolate was established. *G. canis* virus with icosahedron spherical shape and 36 nm in diameter was observed by electron microscope. Conclusion *In vitro* cultivation of *G.canis* trophozoites with GCV is established.

Key words *Giardia canis* Virus *In vitro* culture Modified TYI-S-33 medium

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