中国寄生虫学与寄生虫病杂志 2010 28 (3): 200-204 ISSN: 1000-7423 CN: 31-1248/R

论著

弩巴贝虫病间接ELISA检测方法的建立

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收稿日期 修回日期 网络版发布日期 接受日期

摘要
【摘要】 目的 克隆表达弩巴贝虫诊断抗原基因BC48，建立间接ELISA诊断方法。 方法 从感染弩巴贝虫的毛驴血液中提取虫体基因组DNA，利用PCR技术扩增BC48基因，将其克隆入表达载体pET-28a，转化大肠埃希菌感受态细胞，用异丙基-β-D-硫代半乳糖苷（IPTG）进行诱导表达，用镍柱亲和层析法纯化蛋白。以该蛋白作为诊断抗原建立间接ELISA诊断方法，对反应条件进行优化，并对该方法的特异性和敏感性进行评价。 结果 扩增获得1 272 bp的BC48基因片段。重组表达质粒pET-28a-BC48诱导后获得大小约为M_r 46 000的可溶性融合蛋白，纯化后蛋白浓度为12.98 mg/ml。间接ELISA方法条件优化的结果显示，最佳抗原包被浓度为65 μg/ml，最适血清稀释度为1∶80，该融合蛋白可特异性地与弩巴贝虫感染血清结合，而不与马泰勒虫感染血清及阴性血清反应。使用该方法与镜检法检测17份散养毛驴血清样品，阳性检出率分别为3/17和2/17。 结论 以BC48重组蛋白为抗原建立的间接ELISA方法可用于马属动物弩巴贝虫病的检测。

关键词 弩巴贝虫；BC48；表达；纯化；ELISA
分类号

An Indirect ELISA for the Detection of Babesia caballi in Equine Animals

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Abstract
【Abstract】 Objective To clone and express BC48 gene of Babesia caballi， and to establish an indirect ELISA for the diagnosis of B. caballi in equine animals. Method The genomic DNA of B. caballi was extracted from the infected d*onkey* blood. BC48 gene was amplified by PCR. The PCR product was cloned into expression plasmid pET-28a，and expressed in E. coli BL21 with IPTG induction. The recombinant protein was purified by Ni-NTA affinity chro-matography and was used as a diagnostic antigen to establish an indirect ELISA. The reaction conditions of the indirect ELISA were optimized. Specificity and sensitivity of this method were evaluated. Result BC48 gene of B. caballi was 1 272 bp. The recombinant protein was expressed in E. coli BL21 as a soluble protein with a molecular weight of about Mr 46 000 under induction of IPTG. The concentration of purified protein was 12.98 mg/ml. The best conditions were obtained for the ELISA when the antigen concentration was 65 μg/ml with the serum dilution of 1∶80. The protein specifically reacted with serum from d*onkey* infected by B. caballi， but did not react with serum from d*onkey* infected by Theileria equi （B. equi）. Both ELISA and microscopy were applied to examine 17 d*onkey*s in the field， 3 were positive by ELISA and 2 were found parasite-positive， respectively. Conclusion The indirect ELISA method may be used to detect B. caballi infection in equine animals.

Key words Babesia caballi；BC48；Expression；Purification；ELISA

DOI:

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