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• 调查研究 •

四川省甘孜州棘球蚴病流行区家犬肠道蠕虫感染情况调查^{*}

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【摘要】 目的 调查四川省甘孜州棘球蚴病流行区家犬肠道蠕虫感染情况,为当地人兽共患寄生虫病的防控提供参考。方法 于2023年4月在四川省甘孜州的道孚县、白玉县、德格县和石渠县四个棘球蚴病流行县的18个自然村养犬户院内收集新鲜家犬粪便,提取粪样DNA,通过PCR扩增核糖体的18SrRNA基因、线粒体的cox 1基因和nad 1基因进行虫种鉴定,产物序列在NCBI数据库中进行比对分析以鉴定虫种,统计家犬粪便的蠕虫检出率。结果 本次调查采集家犬粪样道孚县48份,白玉县46份,德格县49份和石渠县47份,190份家犬粪便中有20份检出寄生虫DNA,寄生虫总检出率为10.53%。不同年龄段的犬只蠕虫检出率差异有统计学意义($\chi^2=8.839, P<0.05$),其中5~10岁的犬只蠕虫检出率最高,为10.87%(10/53)。虫种检出率为:狮弓蛔线虫4.74%(9/190),狭首钩刺线虫2.63%(5/190),Chronogaster typica和蛇形毛圆线虫检出率均为1.05%(2/190),Leidynema portentosae,四翼无刺线虫,Diplogasteroides luxuriosae和食道口线虫检出率均为0.53%(1/190)。190份家犬粪便均未检测出棘球绦虫DNA。虫种检出率差异有统计学意义($\chi^2=21.22, P<0.05$)。其中同时检出2种蠕虫的占10%(2/20)。家犬粪便蠕虫检出率德格县为14.29%(7/49),石渠县检出率为12.77%(6/47),道孚县检出率为10.42%(5/48),白玉县检出率为4.35%(2/46)。不同采样点的检出率之间无统计学意义($\chi^2=3.851, P=0.415$)。狭首钩刺线虫在四县的检出率差异有统计学意义($\chi^2=8.439, P<0.05$),德格县的狭首钩刺线虫检出率最高,为8.16%(4/49)。结论 四川省甘孜州棘球蚴病流行区家犬肠道内存在多种蠕虫感染,且部分地区检出率较高,棘球绦虫感染率为0,当地居民依旧存在一定的犬源性寄生虫感染风险。

【关键词】 棘球绦虫流行区;家犬;蠕虫;人兽共患寄生虫病

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Investigation of intestinal worm infection in dogs in Echinococcosis endemic area in Ganzi Prefecture, Sichuan Province

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【Abstract】 **Objective** To investigate the intestinal worm infection of domestic dogs in the Echinococcosis endemic area of Ganzi Prefecture, Sichuan Province, and to provide reference for the prevention and control of zoonotic parasitic diseases in local people. **Methods** In April 2023, fresh dog feces were collected from dog households in 18 natural villages in four Echinococcosis endemic counties of Daofu, Baiyu, Dege and Shiqu, Sichuan Province, and DNA samples were extracted. The 18SrRNA gene of the ribosome, the cox 1 gene of the mitochondria and the nad 1 gene were amplified by PCR to identify the worm species. The product sequences were compared and analyzed in the NCBI database to identify the worm species, and the worm detection rate in the feces of domestic dogs was calculated. Statistical analysis was

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performed using SPSS 26.0 software (SPSS, Chicago, USA). The chi-square test was used to compare ratios or component ratios at a significance level of $\alpha=0.05$. The Fisher exact test is used to compare data that does not meet the requirements of the chi-square test. **Results** 48 dog feces samples were collected from Daofu County, 46 from Baiyu County, 49 from Dege County and 47 from Shiqu County. Parasite DNA was detected in 20 of 190 dog faeces, and the total parasite detection rate was 10.53%. There was significant difference in worm detection rate among dogs of different ages ($\chi^2=8.839, P<0.05$), the highest worm detection rate was 10.87% (10/53) in dogs aged 5 to 10 years. Parasites detected included *Toxascaris leonine* 4.74% (9/190), *Uncinaria stenocephala* 2.63% (5/190), *Chronogaster typical* 1.05% (2/190), *Trichostongylus ophiformis* 1.05% (2/190), *Leidynema portentosae* 1.05% (2/190), *Aspiculuris tetraptera* 1.05% (2/190), *Diplogasteroides luxuriosae* 1.05% (2/190) and *Oesophagostomum nematodes* 0.53% (1/190). No *Echinococcus sp.* DNA was detected in 190 dog faeces. The difference of species detection rate was statistically significant ($\chi^2=21.22, P<0.05$). Two kinds of worms were detected at the same sample in 10% (2/20). The detection rate of fecal worm in domestic dogs was 14.29% (7/49) in Dege County, 12.77% (6/47) in Shiqu County, 10.42% (5/48) in Daofu County and 4.35% (2/46) in Baiyu County. There was no statistical significance between the detection rates of different sampling area ($\chi^2=3.851, P=0.415$). There was significant difference in the detection rate of *U. stenocephala* in four counties ($\chi^2=8.439, P<0.05$), the highest detection rate was 8.16% (4/49) in Dege County.

Conclusion In the endemic areas of Echinococcosis in Ganzi Prefecture, Sichuan Province, there were various worm infections in the intestinal tract of domestic dogs, and the detection rate was high in some areas, although the infection rate of *Echinococcus sp.* was 0. Local residents still have a certain risk of infection of canine parasitic nematodes.

【Key words】 Echinococcus endemic area; domestic dog; worm; zoonotic parasitic disease

我国牧民向来有养犬的传统,主要目的是看家护院以及协助放牧,同时犬是多种人兽共感染寄生虫的主要宿主,常引起人类感染,严重危害人体健康。在我国牧区,家犬的生活环境较差,以及放牧时可能接触到野外环境中的流浪犬或野生动物^[1],增加了其感染肠道寄生虫的风险。根据2014年四川省少数民族地区肠道蠕虫感染现状调查显示,四川省藏族地区和彝族地区带绦虫病高度流行,且牧民为蠕虫感染的重点人群^[2],提示要加强该地区的人群肠道蠕虫病的防控。

四川省甘孜藏族自治州(甘孜州)地处四川省西部,青藏高原东部,幅员面积15.3万km²,下辖1个县级市,17个县,平均海拔3 500 m,人口110.33万,其中藏族占79.4%^[3]。为更好的了解四川省甘孜州家犬肠道蠕虫的感染情况,本研究采用分子生物学技术检测2023年四川省甘孜州部分自然村家犬肠道蠕虫感染情况,为犬的驱虫防治、规范管理和人兽共患寄生虫病防控提供参考。

材料与方法

1 调查点选取

选择四川省甘孜州的道孚县、白玉县、德格县和石渠县四个棘球蚴病流行县的18个自然村作为调查点。

2 样品的采集与处理

在调查村的所有养犬户院内,选择较为新鲜的犬粪进行采集,一犬一份,所有犬只均采集。粪便采集后分别保存于50 mL离心管中并编号。粪样处理前置于-80 °C冷冻1周以灭活蠕虫虫卵感染性。

3 主要试剂和仪器

DNA提取试剂盒[QIAamp Fast DNA Stool Mini Kit (50)]购自德国Qiagen公司,Ex TaqTM PCR预混液(2×)、2%牛血清蛋白、去RNA酶水购自大连市TaKaRa公司,琼脂糖粉购自西班牙Biowest公司,50×TAE缓冲液购自北京市索莱宝科技有限公司。PCR仪(型号CFX96)、凝胶成像仪(型号DocTM XR+)为美国Bio-Rad公司产品,Bio-Rad Gel JY300C电泳仪为北京市北京君意东方电泳设备有限公司产品。

4 PCR扩增动物粪样蠕虫DNA

4.1 粪样处理及DNA提取 粪样过滤后,4 °C、3 000 g离心30 min,弃上清,留沉淀备用。取0.25 g粪样,参考DNA提取试剂盒说明书提取粪样蠕虫DNA。

4.2 PCR扩增 参考Boufana等^[4]的方法,取粪便DNA,分别使用18S核糖体RNA(18SrRNA)基因序列引物^[5],线粒体细胞色素c氧化酶亚基1(cytochrome c oxidase subunit 1, cox 1)基因序列引物^[6]和线粒体NADH脱氢氧化酶亚基(NADH dehydrogenase subunit 1, nad 1)基因序列引物^[4]PCR扩增相应目的基因,检测粪样中蠕虫的感染情况(表1)。PCR反应体系(25 μL):上、下游引物各1 μL,Ex TaqTM PCR Premix(2×)12 μL,牛血清蛋白0.5 μL,模板DNA2 μL,RNase-freeWater 9 μL。PCR反应均在Bio-Rad DNA Engine Dyad PCR仪器上进行,扩增反应条件:94 °C预变性5 min;94 °C变性30 s,退火30 s(退火温度根据引物选择),72 °C延伸30 s,共

通过侵入未受保护的皮肤，幼虫通过皮肤迁移可引起炎症导致皮肤幼虫移行症 CLM^[10]，在欧洲，包括英国、德国、意大利和塞尔维亚都有过人类 CLM 的病例报道^[11]。我国的三江源区域^[9]，青海省囊谦县^[12]，大兴安岭地区^[13]均在犬粪便中检测出狭首钩刺线虫，本调查中，德格县的狭首钩刺线虫检出率最高，这提示德格县应该加强人群和犬只感染狭首钩刺线虫的筛查，做好犬只驱虫，以及人群健康教育等防控工作。有关四川省甘孜州家犬感染狭首钩刺线虫的报道较少，其具体感染情况还需要进一步研究。

除上述线虫外，本次调查中还发现了一些非犬科动物寄生线虫。蛇形毛圆线虫一种人兽共患寄生虫，常见于反刍动物的肠道中，是牛羊体内最常见的毛圆属线虫。人体感染是由于摄入被其第三期感染性幼虫污染的食物^[14]。食道口线虫是麂子胃肠道常见寄生虫^[12]。*L. portentosae* 和 *D. luxuriosae* 为蟑螂等昆虫肠道寄生虫^[12,15]。四翼无刺线虫是实验室感染动物模型的重要寄生线虫^[16]。这些寄生虫的检出，可能与家犬的捕食习性有关。

本调查以家犬为研究对象，和其他对甘孜州野外犬科动物的肠道寄生虫调查^[17]结果(36.43%，47/129)对比，检出率差异有统计学意义($\chi^2 = 31.083, P < 0.05$)，表明家犬肠道寄生虫检出率低于野外犬科动物，这可能是由于家犬做到了驱虫和拴养，有效降低了家犬肠道寄生虫的感染。5~10岁的犬只粪便蠕虫检出率最高，和 Duncan 等^[18]的调查结果一致，表明应对有5~10岁犬只的牧民肠道寄生虫筛查以及健康教育，并加强对5~10岁犬只的驱虫管理。

四川省甘孜州作为棘球蚴病流行区，根据2018-2022年四川省甘孜州棘球蚴病监测结果分析^[19]显示，截至2022年，甘孜州人群患病率为0.01%，低于2016年^[20]的0.158%，甘孜州2018-2022年犬的棘球绦虫感染率显著降低，提示甘孜州棘球蚴病防治工作取得巨大成效。在本调查中，家犬粪便棘球绦虫检出率为0，进一步表明当地棘球蚴病防治效果突出，但家犬肠道蠕虫检出率高于大兴安岭地区^[13](8.42%，17/202)，新疆维吾尔自治区呼图壁县^[21](7.87%，14/178)等地区，表明家犬粪便未检测出棘球绦虫可能主要是因为当地进行棘球蚴病的健康教育，居民不再向犬只投喂家畜内脏以及拴养犬只，阻断了棘球绦虫的传播循环。但由于棘球蚴病流行区犬驱虫药为吡喹酮，主要针对绦虫和吸虫的驱除，可能对于线虫的驱虫效果不佳^[22]，这使得人群暴露在其他人兽共患肠道线虫的风险下。阿苯达唑为一高效低毒的广谱驱虫药，临幊上对线虫敏感，绦虫、吸虫也有较强作用^[22]，因此建议相关部门是否可以将阿苯达唑纳入当地犬驱虫药

物，和吡喹酮联合用药，增加驱虫的广谱性，并定时对犬驱虫效果进行评估，改良驱虫手段以保证驱虫效果，从而保障人畜健康。

综上所述，四川省甘孜州棘球蚴病流行区家犬肠道内存在多种蠕虫感染，且部分地区检出率较高，棘球绦虫感染率为0，当地居民依旧存在一定的犬源性寄生线虫感染风险。其中德格县家犬狭首钩刺线虫检出率最高，犬只年龄是犬只感染肠道蠕虫的影响因素，5~10岁的犬只肠道蠕虫感染率最高，建议加强重点犬只的管理以及建议使用阿苯达唑-吡喹酮联合用药以增强驱虫效果。由于本次调查样本量相对较小，存在影响家犬感染的因素被掩盖的可能性，建议后续研究应结合实际情况适当增加样本量，并可以选择不同月份、分批次调查，来分析时间(季节)对于家犬肠道蠕虫感染的影响，为开展科学有效的犬源性寄生虫病防控提供参考依据。

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