

伊维菌素对长角血蜱生长发育及生殖的影响*

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摘要 【目的】通过分析宿主皮下注射伊维菌素对长角血蜱生长发育及生殖的影响, 进而评价伊维菌素对长角血蜱的防治效果。【方法】以新西兰白兔为宿主, 皮下注射不同剂量伊维菌素, 观察幼蜱、若蜱及雌蜱的叮咬率、死亡率、吸血期、饱血体重、产卵量和孵化率等生物学参数, 分析伊维菌素对长角血蜱生殖和生长发育的影响。【结果】伊维菌素对长角血蜱各期蜱虫的叮咬率均无显著影响。与对照组相比, 伊维菌素可显著增加幼蜱和若蜱死亡率, 延长若蜱和雌蜱的吸血期并降低其饱血体重, 降低雌蜱产卵量及卵孵化率, 抑制卵巢及卵的发育。【结论】宿主皮下注射伊维菌素能够显著抑制长角血蜱的发育及生殖, 可作为防治长角血蜱的候选杀虫剂。

关键词 长角血蜱; 伊维菌素; 生殖; 发育; 蜱类防治

The effect of ivermectin on the development and reproduction of *Haemaphysalis longicornis*

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Abstract [Objectives] To investigate the effectiveness of injected ivermectin for controlling infestation with *Haemaphysalis longicornis*. [Methods] Different doses of ivermectin were injected subcutaneously into New Zealand white rabbits infected with *H. longicornis*. Biological parameters including biting rate, mortality, feeding period, engorgement weight, female fecundity, hatchability, and changes in ovarian morphology and eggs, were measured to determine the effect of ivermectin on the reproduction and development of *H. longicornis*. [Results] Treatment with ivermectin had no significant effect on the biting rate of larvae, nymphs or adult females, but did significantly increase the mortality of larvae and nymphs, significantly prolonged the feeding period, and reduced the engorged weight of both nymphs and female ticks. Ivermectin also significantly reduced the number of eggs laid per female, hatchability, and inhibited ovarian development. [Conclusion] Injection with ivermectin can effectively suppress the development and reproduction of *H. longicornis* and could be an effective treatment for controlling this parasite.

Key words *Haemaphysalis longicornis*; ivermectin; development; reproduction; tick control

长角血蜱 *Haemaphysalis longicornis* 隶属硬蜱科 (Ixodidae) 血蜱属 (*Haemaphysalis*), 是一种专性吸血的体外寄生虫, 主要分布于中国、韩国、日本、新西兰、澳大利亚和美国 (Zhang et al., 2018; Tufts et al., 2019)。其发育过程经卵、幼蜱、若蜱和成蜱四个阶段, 且后三个阶段皆寄生

于宿主体表吸血。长角血蜱寄生期可将多种病原体传播至宿主, 包括泰勒虫属 (*Theileria*) 卵形巴贝斯虫 *Babesia ovata*、吉氏巴贝斯虫 *Babesia gibsoni*、立克次体属 (*Rickettsia*) 无浆体属 (*Anaplasma*)、螺旋体 (*Treponema*) 和病毒等 (Luo et al., 2019; Zhang et al., 2019;

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Wang et al., 2019), 给人类健康及畜牧业发展造成极大危害 (Abreu et al., 2017)。因此, 研究其防治具有重要的医学、流行病学和经济学意义。目前, 蜱类防治措施主要包括四个方面: 化学防治、生物防治、免疫防治及遗传防治, 其中化学防治因其效果持久且价格低廉是目前应用最广泛的防治措施 (郝雪峰等, 2008)。

伊维菌素 (Ivermectin) 是人工合成的大环内酯类化合物 (Campbell et al., 1983), 对寄生虫特别是线虫和节肢动物均有较好的防治效果 (Cho, 2011; Page, 2019)。伊维菌素可与靶虫细胞上谷氨酸-氯离子通道受体结合, 增加细胞膜对氯离子的通透性, 减少兴奋性递质的释放, 导致虫体麻痹、死亡 (Pohl et al., 2011)。此外, 研究证实伊维菌素可使寄生虫体内 ATP 酶活性下降, 影响钠钾泵的跨膜运输作用, 最终致虫体死亡 (Shu et al., 2000)。通过浸泡或皮下注射伊维菌素溶液可有效防治环须扇头蜱 *Rhipicephalus annulatus* (El-Bahy et al., 2015)、微小扇头蜱 *Rhipicephalus microplus* (Pereira, 2009; Davey et al., 2010)、小亚璃眼蜱 *Hyalomma anatolicum anatolicum* (Sajid et al., 2009); Doan 等 (2013) 研究发现阿维菌素类药物可降低长角血蜱若蜱及雌蜱的饱血体重, 但此类药物对长角血蜱各生长发育阶段生物学指标的影响未见报道。

本研究以长角血蜱为研究对象, 新西兰白兔 *Nuova Zelanda conigli bianchi* 为宿主, 皮下注射不同剂量伊维菌素, 观察幼蜱、若蜱及雌蜱生长发育相关的参数: 叮咬率、死亡率、吸血期、饱血体重; 同时观察雌蜱生殖相关的参数: 产卵量、孵化率及卵巢、卵的形态变化, 分析伊维菌素对长角血蜱生殖和发育的影响, 系统评价了宿主皮下注射伊维菌素对长角血蜱的防治效果, 明确了伊维菌素有效的注射剂量, 为有效开展蜱类的防治提供了科学依据。

1 材料与方法

1.1 实验动物

长角血蜱采自河北省小五台国家自然保护

区, 于实验室内传代繁殖。寄生期于新西兰白兔耳上饱血, 非寄生期于温度 (25 ± 1) , 相对湿度 75%, 光照周期为 16L:8D 的恒温培养箱内培养。

1.2 实验方法

幼蜱组, 随机选取 6 只健康新西兰白兔, 1 只不注射药物为空白对照, 其余 5 只分别按 0.1、0.2、0.4、0.6、0.8 mg/kg 浓度背部皮下注射伊维菌素, 药物注射 24 h 后, 取 600 头饥饿期幼蜱放至无蜱叮咬史的兔耳上, 每只兔耳 50 头。饲喂 3 d 后, 再次对家兔注射相同剂量的伊维菌素, 以维持其血药浓度, 每天观察记录兔耳上幼蜱生长发育相关的参数 (1) 叮咬率: 为叮咬于兔耳的幼蜱数/饲喂于兔耳的幼蜱数 (2) 死亡率: 为叮咬后死亡的幼蜱数/叮咬于兔耳的幼蜱数 (3) 饱血体重: 幼蜱饱血后主动从兔耳皮肤脱落, 此时称重, 即为饱血体重 (4) 吸血期: 从幼蜱叮咬于兔耳皮肤开始, 至其饱血脱落结束。设置 3 个平行试验组, 重复上述试验。若蜱组, 每只兔耳 50 头若蜱; 成蜱组, 每只兔耳 40 头成蜱, 其余实验方法及检测参数与幼蜱组相同。此外, 雌蜱还需观察记录生殖相关的参数 (1) 产卵量: 将饱血脱落的雌蜱置于编号的培养皿中, 于实验室培养箱中恒温培养, 记录每天产卵数量, 计算总和 (2) 孵化率: 为孵出幼蜱的数量/卵的数量 (3) 体视镜下观察卵巢及卵形态学变化。具体实验流程见图 1。用 Excel 和 STATISTICA 软件完成统计分析, 对叮咬率、死亡率、饱血体重、吸血期、产卵量及孵化率进行 *t*-检验 (*t*-test), 差异显著水平为 $P<0.05$ 。

2 结果与分析

2.1 伊维菌素对长角血蜱幼蜱生长发育的影响

与对照组相比, 伊维菌素各剂量对幼蜱叮咬率均无显著影响 ($t_{0.1}=0.476$, $t_{0.2}=1.145$, $t_{0.4}=0.897$, $t_{0.62}=0.782$, $t_{0.8}=0.092$, $P>0.05$) (图 2: A)。0.1 mg/kg 剂量组的死亡率与对照组无显著差异 ($t_{0.1}=0.365$, $P>0.05$), 其余剂量组死亡率

均为 100%，表明伊维菌素可显著增加长角血蜱幼蜱的死亡率（图 2：B）；实验组幼蜱叮咬后 1-2 d 内全部死亡，故无法观察吸血期及测量饱血体重变化。

2.2 伊维菌素对长角血蜱若蜱生长发育的影响

与对照组相比，伊维菌素各剂量对若蜱叮咬率均无显著影响 ($t_{0.1}=0.301$, $t_{0.2}=0.924$, $t_{0.4}=1.560$, $t_{0.6}=0.191$, $t_{0.8}=1.753$, $P>0.05$) (图 3：A)。0.1 mg/kg 剂量组的死亡率与对照组无显著差异 ($t_{0.1}=0.153$, $P>0.05$)，0.2、0.4、0.6、0.8 mg/kg 剂量组死亡率分别为 64.4%、82.7%、99.73% 及

99.83% ($t_{0.2}=8.569$, $t_{0.4}=18.01$, $t_{0.6}=73.46$, $t_{0.8}=73.92$, $P<0.05$)，表明伊维菌素可显著增加长角血蜱若蜱的死亡率，且在 0.6 mg/kg 以内呈剂量相关性（图 3：B）。

0.1 mg/kg 剂量组吸血期天数与对照组无显著差异 ($t_{0.1}=1.667$, $P>0.05$)，0.2、0.4 mg/kg 剂量组分别使吸血期延长 1.1 d、1.6 d ($t_{0.2}=4.468$, $t_{0.4}=13.27$, $P<0.05$)，表明伊维菌素可显著延长长角血蜱若蜱的吸血期（图 3：C）。0.6 mg/kg 与 0.8 mg/kg 剂量组若蜱全部死亡，故无法观察吸血期变化。

0.1 mg/kg 剂量组的饱血体重与对照组无显

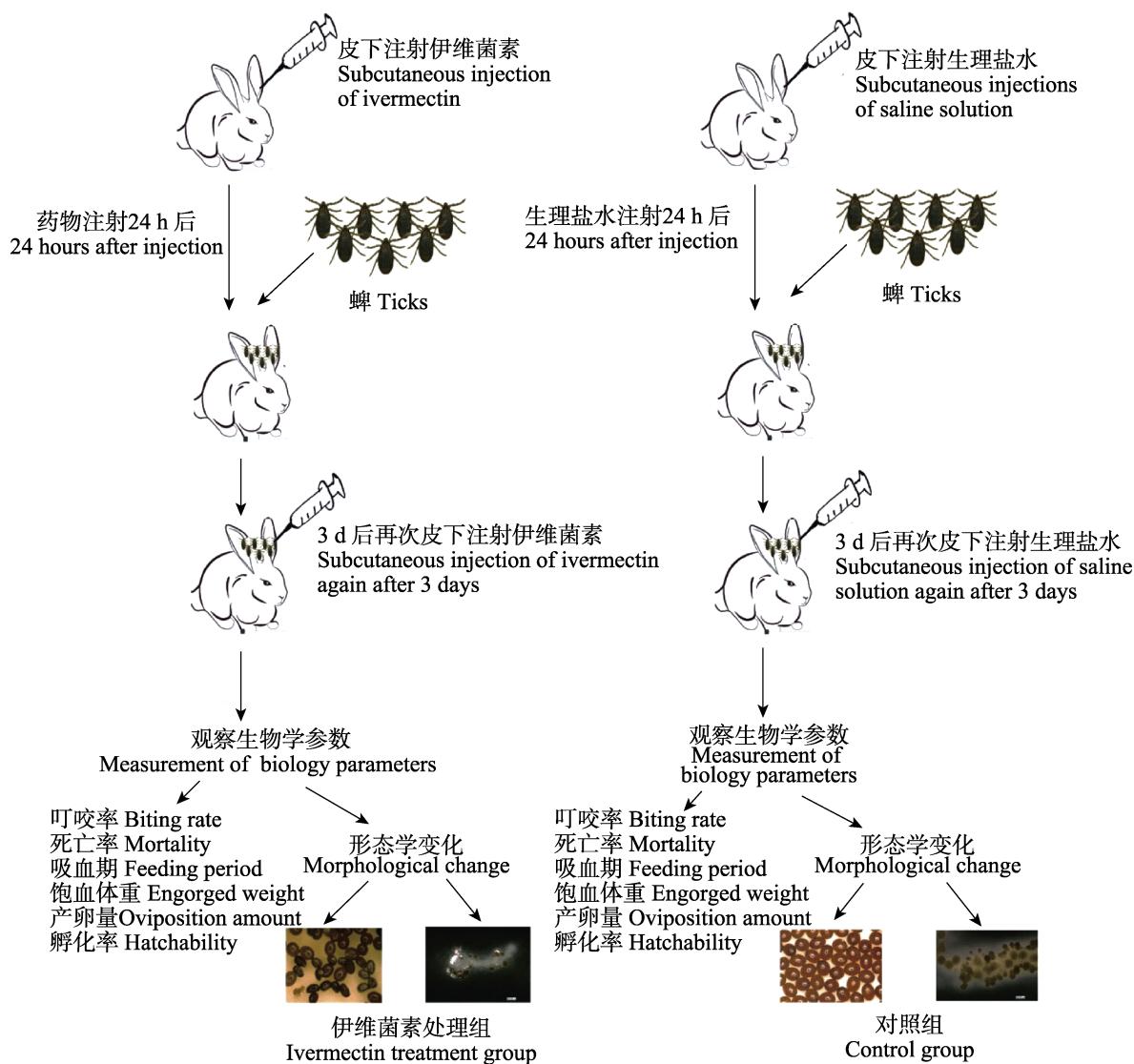


图 1 实验流程图

Fig. 1 Experimental design outline, represented as a flow chart

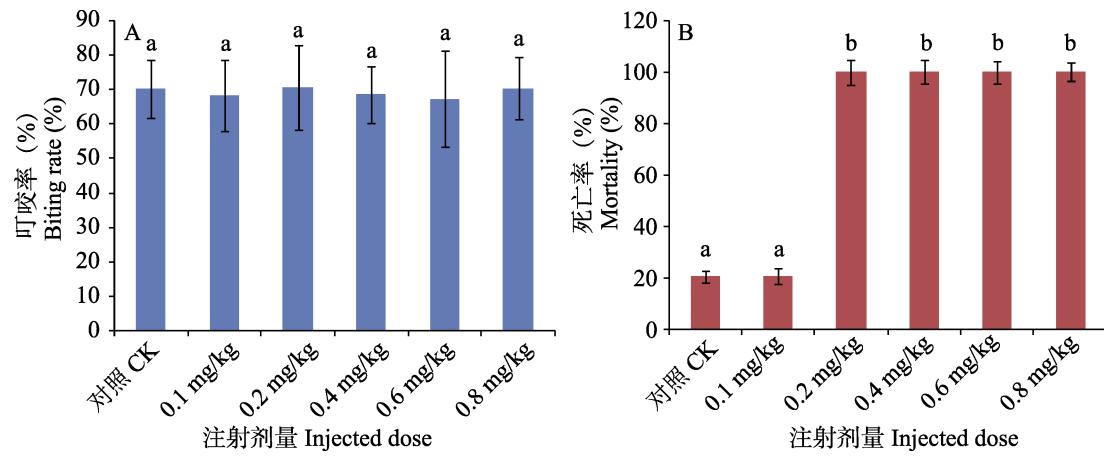


Fig. 2 The effects of different doses ivermectins on biological parameters of larvae *Haemaphysalis longicornis*

A. 叮咬率变化 ; B. 死亡率变化。

A. The changes of biting rate; B. The changes of mortality.

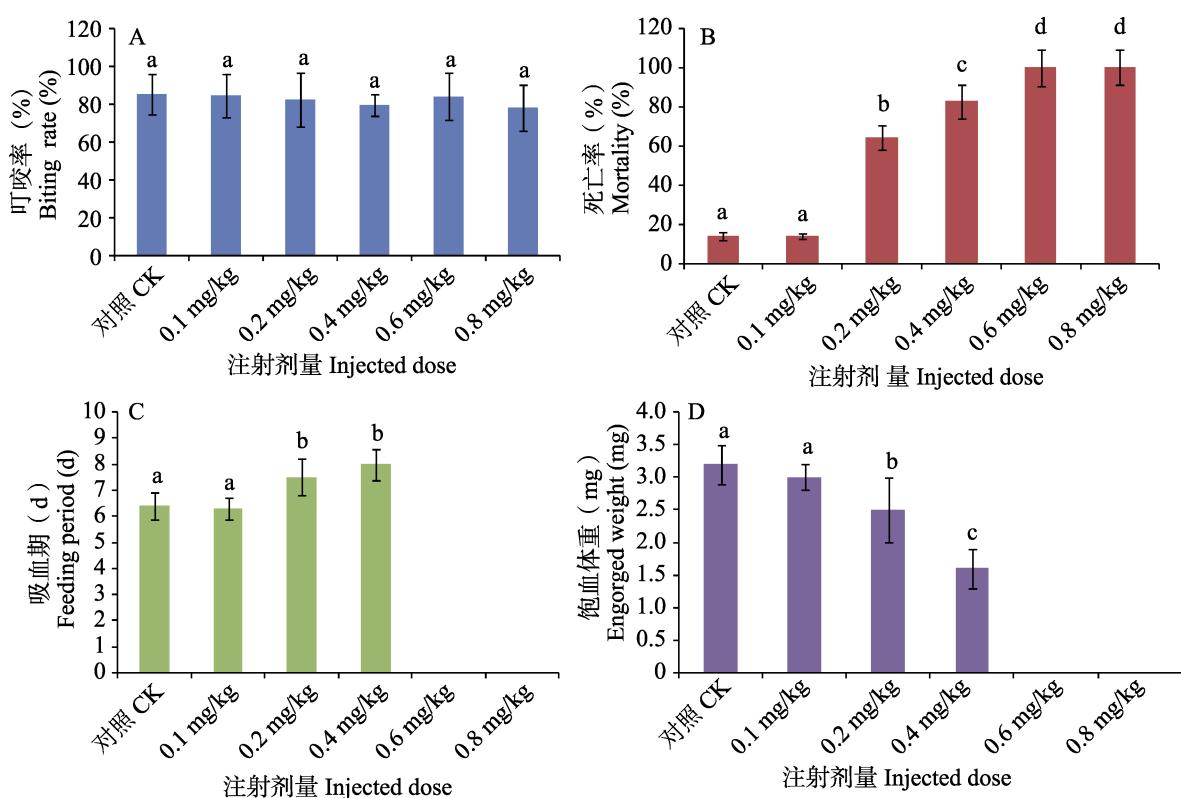


Fig. 3 The effects of different doses ivermectins on biological parameters of nymphs *Haemaphysalis longicornis*

A. 叮咬率变化 ; B. 死亡率变化 ; C. 吸血期天数变化 ; D. 饱血体重变化。

A. The changes of biting rate; B. The changes of mortality; C. The changes of feeding period;
D. The changes of engorgement weight.

著差异($t_{0.1}=1.313, P>0.05$)。0.2 mg/kg、0.4 mg/kg 剂量组分别使饱血体重降低 21.8% 及 50%，

($t_{0.2}=4.245, t_{0.4}=3.115, P<0.05$)，表明伊维菌素可显著降低长角血蜱若蜱的饱血体重，且在

0.4 mg/kg 以内呈剂量相关性(图 3:D), 0.6 mg/kg 与 0.8 mg/kg 剂量组若蜱全部死亡, 故无法观察饱血体重变化。

2.3 伊维菌素对长角血蜱雌蜱生长发育及生殖的影响

与对照组相比, 伊维菌素各剂量对雌蜱叮咬率($t_{0.1}=0.549$, $t_{0.2}=1.016$, $t_{0.4}=0.074$, $t_{0.6}=0.533$, $t_{0.8}=0.451$, $P>0.05$)、死亡率($t_{0.1}=0.854$, $t_{0.2}=0.394$, $t_{0.4}=1.610$, $t_{0.6}=0.259$, $t_{0.8}=1.379$, $P>0.05$)均无显著影响(图 4:A, B)。

0.1 mg/kg 剂量组吸血期天数与对照组无显著差异($t_{0.1}=0.113$, $P>0.05$), 0.2、0.4、0.6、0.8 mg/kg 剂量组分别使吸血期延长 0.7、1.4、1、2.6 d($t_{0.2}=5.956$, $t_{0.4}=3.837$, $t_{0.6}=3.222$, $t_{0.8}=9.216$, $P<0.05$), 表明伊维菌素可显著延长长角血蜱雌蜱的吸血期, 且在 0.4 mg/kg 以内呈剂量相关性(图 4:C)。

0.1 mg/kg 剂量组的饱血体重与对照组无显

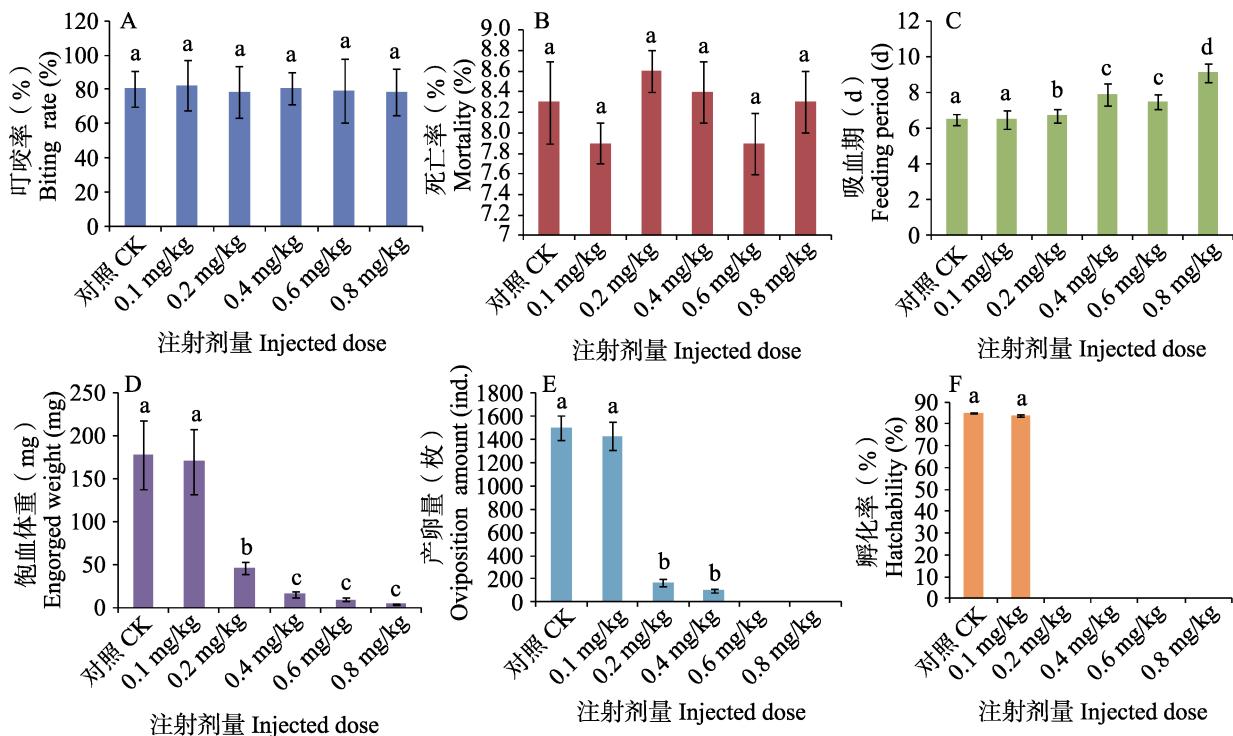


图 4 宿主皮下注射不同剂量伊维菌素长角血蜱雌蜱生物学参数变化
Fig. 4 The effects of different doses ivermectins on biological parameters of female ticks *Haemaphysalis longicornis*

A. 叮咬率变化 ; B. 死亡率变化 ; C. 吸血期天数变化 ; D. 饱血体重变化 ; E. 产卵量变化 ; F. 孵化率变化。
A. The changes of biting rate; B. The changes of mortality; C. The changes of feeding period; D. The changes of engorgement weight; E. The changes of oviposition amount; F. The changes of hatchability.

著差异($t_{0.1}=0.894$, $P>0.05$), 0.2、0.4、0.6、0.8 mg/kg 剂量组分别使饱血体重降低 73.7%、91.1%、94.5%、97.4% ($t_{0.2}=5.620$, $t_{0.4}=6.409$, $t_{0.6}=6.619$, $t_{0.8}=6.801$, $P<0.05$), 表明伊维菌素可显著降低长角血蜱雌蜱饱血体重, 且在 0.4 mg/kg 以内呈剂量相关性(图 4:D)。

0.1 mg/kg 剂量组产卵量与对照组无显著差异($t_{0.1}=0.097$, $P>0.05$), 0.2 mg/kg、0.4 mg/kg 剂量组分别使雌蜱产卵量减少 88.8%、93.3% ($t_{0.2}=16.73$, $t_{0.4}=17.34$, $P<0.05$), 0.6 mg/kg 与 0.8 mg/kg 剂量组产卵量均为 0, 表明伊维菌素可显著降低长角血蜱雌蜱产卵量(图 4:E)。

0.1 mg/kg 剂量组卵孵化率与对照组无显著差异($t_{0.1}=0.679$, $P>0.05$), 其余剂量组孵化率均为 0, 表明伊维菌素可显著降低长角血蜱卵的孵化率(图 4:F)。

0.1 mg/kg 剂量组卵巢及卵的形态与对照组无显著差异, 其余剂量组卵巢发育显著滞后, 卵泡数量显著减少, 卵皱缩变形(图 5)。

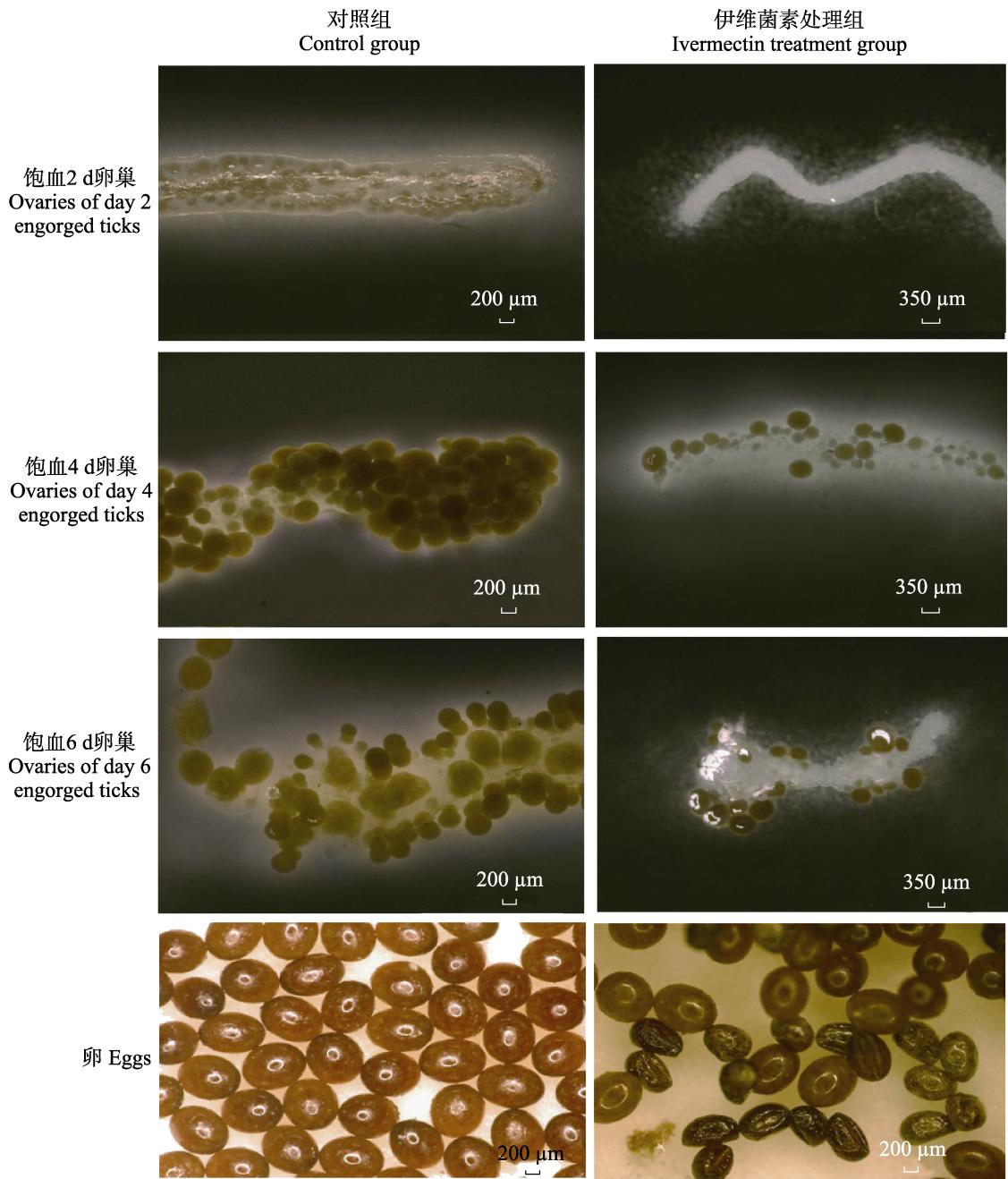


图 5 宿主皮下注射 0.4 mg/kg 伊维菌素长角血蜱雌蜱卵巢及卵的变化
Fig. 5 The effects of ivermectin (0.4 mg/kg) on ovary and egg of female ticks *Haemaphysalis longicornis*

左侧为空白对照组，右侧为 0.4 mg/kg 伊维菌素处理组。

The blank control group is on the left, and the treatment group with 0.4 mg/kg ivermectin is on the right.

3 讨论

蜱携带多种病原体，给人类健康及畜牧业带来极大危害。据联合国粮农组织统计，每年硬蜱给全球畜牧业造成的损失高达 70 亿美元 (Suarez *et al.*, 2018)。目前，药物灭蜱仍是防治蜱的主

要途径，常用药物包括有机磷制剂、拟除虫菊酯类化合物及抗生素类药物，采用方式多为宿主体表喷洒或涂抹 (Lu *et al.*, 2015; Nandi *et al.*, 2018)。大规模喷洒灭蜱药物导致药物使用剂量增加，加速了虫体抗药性的产生，缩短了防效作用间隔，同时对生态环境造成污染 (Rodríguez-

Hidalgo *et al.*, 2017; Khangembam *et al.*, 2018)。因此,合理的施用方式及剂量已成为提高化学防治效果的重要手段。本研究探讨了宿主皮下注射灭蜱药物防治长角血蜱的效果,此种给药方式能有效减少药物使用剂量及环境污染且易在畜牧业中开展(George and Davey, 2004)。

伊维菌素早期多用于线虫防治。由于使用简便、对外寄生虫效果显著及其他杀蜱剂耐药性增加,伊维菌素用于微小扇头蜱的防治并取得良好的效果(Cramer *et al.*, 1988; George and Davey, 2004)。研究证实羊、兔伊维菌素中毒剂量为3 mg/kg,牛中毒剂量为6 mg/kg,故本研究设置浓度梯度均远低于其中毒剂量(邢一丹等,2008)。本研究分析了皮下注射伊维菌素对长角血蜱各发育阶段的叮咬率、死亡率、吸血期、饱血体重及对雌蜱的产卵量、孵化率、卵巢及卵的形态的影响。结果显示伊维菌素对长角血蜱各发育阶段的叮咬率均无显著影响,提示该药物对长角血蜱无趋避作用;伊维菌素显著增加幼蜱、若蜱的死亡率,0.2 mg/kg 伊维菌素即可使幼蜱死亡率达100%,且目前未见伊维菌素对幼蜱防治效果的报道;它还可显著延长若蜱、雌蜱吸血期且降低其饱血体重:吸血期延长提示蜱虫吸血过程受抑制,进而影响其发育;它还可抑制雌蜱卵巢及卵的发育。以上结果表明伊维菌素可对长角血蜱幼蜱、若蜱及雌蜱的生长发育及生殖产生显著的影响,可有效防治长角血蜱。根据实验结果,幼蜱的防治推荐宿主皮下注射剂量为0.2 mg/kg,若蜱、成蜱均为0.4 mg/kg。野外环境中,长角血蜱幼蜱、若蜱及成蜱3个阶段的发生存在重叠现象,但有明显的季节消长变化,即幼蜱、若蜱及成蜱高峰期出现在不同时间节点:幼蜱数量高峰期出现在9月上旬,若蜱高峰期出现在4月下旬,成蜱高峰期出现在7月中旬(Zheng *et al.*, 2012),因此可针对幼蜱、若蜱及成蜱集中出现时期于宿主皮下注射伊维菌素的最适剂量进行蜱的防治。

Doan等(2013)研究显示0.2 mg/kg 伊维菌素可使长角血蜱若蜱饱血体重下降20%,本研究

结果与这一结果相似。El-Bahy等(2015)将环须扇头蜱成蜱浸泡在50 ng/mL 伊维菌素溶液中,1 h后成蜱死亡率为51%,2 h后死亡率为95%,且防治效果较溴氰菊酯、地嗪农显著;Sajid等(2009)将小亚璃眼蜱成蜱浸泡于13、26、39 μg/mL 伊维菌素溶液中5 min,其死亡率分别为58%、70%及86.7%;而本研究结果显示伊维菌素对雌蜱死亡率无显著影响,这可能与给药方式及药物剂量差异有关。Davey等(2010)研究发现皮下注射0.63 mg/kg 伊维菌素后,微小扇头蜱雌蜱饱血体重降低90%;而本研究结果显示0.6 mg/kg 伊维菌素使雌蜱饱血体重降低94.5%,提示伊维菌素对长角血蜱效果优于微小扇头蜱。本研究在实验室条件下系统地探讨了伊维菌素对长角血蜱的防治效果,并为防治蜱虫推荐了注射剂量,减少了宿主体内的药物残留,为蜱类防治提供了基础性资料。关于野外环境条件下,宿主皮下注射伊维菌素最适剂量后蜱的防治效果亟待后续研究证实。

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