



## 东方蜜蜂微孢子虫传染病学分析

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**摘要:** 东方蜜蜂微孢子虫病是一种由东方蜜蜂微孢子虫(*Nosema ceranae*)引起的蜜蜂传染病, 已经蔓延到全球。蜜蜂感染东方蜜蜂微孢子虫后会导致早衰、哺育能力下降、生产力和繁殖能力降低, 严重时可直接导致蜂群瓦解。本文从传染病学角度出发, 对近10年东方蜜蜂微孢子虫病原学、流行病学和防治方法等方面进行总结, 以此提高对微孢子虫的认识, 为微孢子虫防治提供新思路。

**关键词:** 东方蜜蜂微孢子虫, 传染病学分析, 防治方法, 蜜蜂

在过去半个世纪里, 全世界作物种植面积增加了约 25%, 其中相当多一部分是依靠授粉的虫媒花植物<sup>[1]</sup>。蜜蜂是全球范围内最重要的授粉者<sup>[2-3]</sup>, 也是目前唯一被人类大规模饲养, 并能够在短时期内完成对密集开花的高产作物进行授粉的昆虫<sup>[4]</sup>。蜂群的快速衰减为农业生产带来严重威胁<sup>[5-6]</sup>, 这一点在北美的表现尤为突出<sup>[7-8]</sup>。造成蜂群数量下降的因素是多方面的, 除农药的使用、环境的变化所带来的不利因素外, 越来越多的研究证实寄生虫及病原体的感染是造成蜂群死亡的主要因素<sup>[9-11]</sup>。东方蜜蜂微孢子虫病是

由东方蜜蜂微孢子虫引起的一种蜜蜂消化道传染病<sup>[12]</sup>, 主要通过被污染的水源和食物进行蜂群内及蜂群间的互相传播<sup>[13-15]</sup>。蜜蜂在感染后表现出定向和归巢能力降低、加速老龄化、蜂群抵抗力和哺育能力下降、采集蜂过早死亡<sup>[16-20]</sup>等现象。

1996 年, 东方蜜蜂微孢子虫首次在北京的东方蜜蜂肠道内被发现和命名<sup>[21]</sup>。2003 年以来, 美国、巴西、墨西哥、芬兰、澳大利亚及非洲等多个国家或地区均有东方蜜蜂微孢子虫感染蜜蜂的报道<sup>[22-26]</sup>, 此外熊蜂(*Bombus*)等多个蜂种均可作为东方蜜蜂微孢子虫的寄生宿主<sup>[15]</sup>。

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## 1 病原学

东方蜜蜂微孢子虫病的病原属于微孢子虫科(*Nosematidae*)微孢子虫属(*Nosema*)的成员, 被命名为东方蜜蜂微孢子虫。东方蜜蜂微孢子虫属于单细胞真核生物, 不能在宿主细胞外繁殖, 没有经典的线粒体, 但携带有由线粒体衍生的细胞器, 称为线粒体残迹(mitosome), 通过利用宿主的能量物质完成自身的快速繁殖<sup>[27]</sup>。成熟的孢子为椭圆形, 可在 400 倍光学显微镜下观察到, 其平均大小为  $4.5\ \mu\text{m} \times 2.4\ \mu\text{m}$ , 孢壁主要成分为几丁质和糖蛋白<sup>[28]</sup>。在扫描电镜下可以观察到东方蜜蜂微孢子虫孢壁上具有粗糙褶皱(图 1); 透射电镜下可测得孢壁厚度为 137–183 nm, 由内向外可分为刺突状外层、电子透明薄层和纤维性内层, 其中电子透明层较厚, 从孢壁整体上看前端孢壁较薄, 只有约 36 nm, 是孢子抛出极丝的位置, 孢子前端具有固根盘结构, 后端是具有空泡结构的后极泡, 内质网在核的两端依次缠绕。极丝具有 4 层结构, 在核外盘绕 20–23 圈, 螺旋倾角为  $55^\circ$ – $60^\circ$ <sup>[29]</sup>。

永久的蜜蜂细胞培养系和蜜蜂病原体细胞的培养模型的缺乏严重制约了蜜蜂病原体与靶细胞之间的分子和细胞相互作用的研究进展<sup>[12]</sup>。为了克服这一障碍, Gisder 等基于从吉普赛蛾卵巢建立的异源鳞翅目细胞系 IPL-LD-65Y 开发了专性细胞内蜜蜂病原体东方蜜蜂微孢子虫的细胞培养模型, 为东方蜜蜂微孢子虫在蜜蜂细胞内繁殖周期提供了新的见解<sup>[12,30]</sup>。与 IPL-LD-65Y 悬浮细胞共同孵育萌发的东方蜜蜂微孢子虫显示, 萌发的孢子挤出极管, 从远处击中目标细胞, 然后将孢子质注入宿主细胞<sup>[31]</sup>。此后不久, 感染细胞中的孢子质变为球形小体。对细胞内生命周期的时程分析表明: 接种东方蜜蜂微孢子虫 16 h 后, 注入的孢子质发育成东方蜜蜂微孢子虫的营养阶段, 形成一个纺锤形分生组织。在随后的 4 h, 第一对纺锤形的分生组织开始出现。然后, 这些纺锤形分生孢子成倍增加, 直到第一个凝聚结构的形成。在感染细胞的 48–72 h 期间可以检测到发育成初生孢子和许多对纺锤形分生粒。初生孢子为圆形, 而不是环境孢子的椭圆形<sup>[32]</sup>, 成熟孢子在感染细胞大约 96 h 后可以检测到(图 2)。

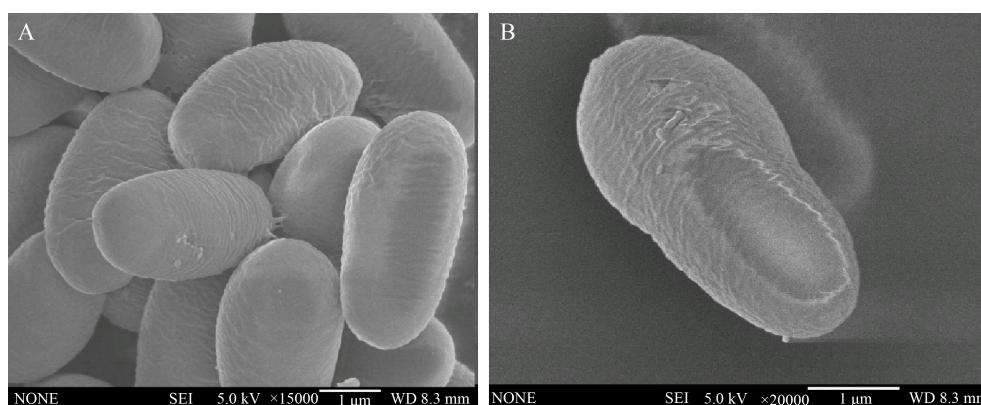


图 1. 扫描电镜下东方蜜蜂微孢子虫的外部形态特征<sup>[29]</sup>

Figure 1. The external morphology of *Nosema ceranae* by scanning electronic micrographs<sup>[29]</sup>. A: external morphology of multiple spores; B: external morphology of a single spore.

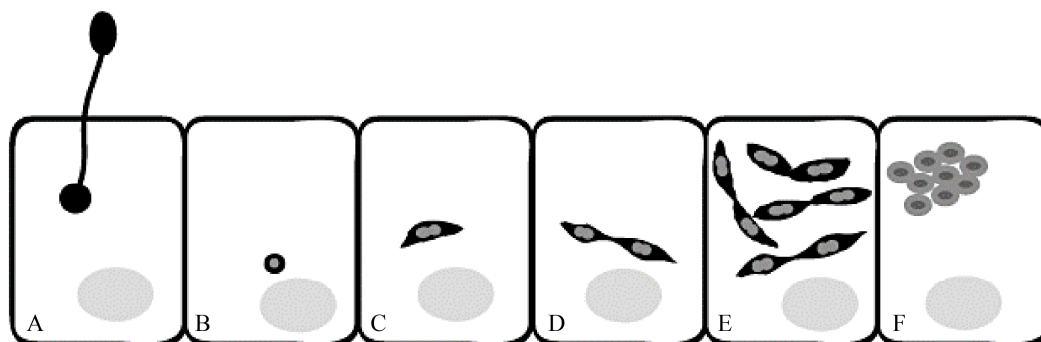


图 2. 东方蜜蜂微孢子虫繁殖周期示意图<sup>[12]</sup>

Figure 2. Schematic representation of the early events in the life cycle of *Nosema ceranae*<sup>[12]</sup>. A: membrane of the target cell followed by injection of the sporoplasm into the host cell; B: the sporoplasm appears as small spherical body in the host cell; C: develops into a spindle-shaped meront; D: which begins to divide giving rise to paired meronts; E: these pairs of meronts then undergo several rounds of cell division; F: they separate and develop into round to oval sporonts, which are condensed and characterized by a thickened plasma membrane.

## 2 流行病学

### 2.1 易感动物

东方蜜蜂微孢子虫具有感染蜂种多、范围广的特点<sup>[15]</sup>。成年蜜蜂在各阶段对本病均易感。在同一蜂群中，新出房蜜蜂体内几乎无东方蜜蜂微孢子虫，采集蜂感染量最高，其次为哺育蜂<sup>[33]</sup>。利用人工方法对蜜蜂接种东方蜜蜂微孢子虫，结果表明，蜜蜂在出房 1 d 内最易感染东方蜜蜂微孢子虫，在出房 5 d 后对微孢子虫染不敏感<sup>[34]</sup>。东方蜜蜂微孢子虫对蜜蜂来说是具有高度特异性的消化道寄生物，在感染后的第 3 天，利用 400 倍光学显微镜可在中肠上皮细胞、排泄物和分泌物中观察到成熟的孢子<sup>[18]</sup>。

### 2.2 传染源及传播途径

东方蜜蜂微孢子虫主要通过直接接触传播和间接接触传播两个途径。在蜂群内部，由于蜜蜂具有交哺的生物学特性，患病蜂在交哺行为发

生过程中将东方蜜蜂微孢子虫传给了健康蜜蜂<sup>[35-36]</sup>。在外界环境中，东方蜜蜂微孢子虫通过被病蜂排泄物、分泌物污染的蜂粮和水源进行传播。此外 Sulborska 等<sup>[37]</sup>曾报道，干燥的粪便及某些生物气溶胶内携带的东方蜜蜂微孢子虫可以借助空气进行传播。显然在饲养管理中不恰当的并群和盗蜂现象的产生会加速东方蜜蜂微孢子虫在蜂群间的传播。卫生条件差、蜂群营养状况不佳等不良的饲养管理条件会提高该病的发病率和死亡率。一般情况下，弱群在感染后所表现出的危害性比强群更明显，这主要是因为弱群抵抗力和免疫力更弱。

### 2.3 流行特点

Martin-Hernandez<sup>[38]</sup>在 2005 年对西班牙感染东方蜜蜂微孢子虫的蜂群感染率进行统计分析，结果显示东方蜜蜂微孢子虫的感染无季节特异性。但在 2008 年，Higes 等<sup>[39]</sup>对采集蜂和哺育蜂的感染率以及蜜蜂中肠内孢子载量进行检测，发

现蜂群在感染后第二年秋季,微孢子感染率和蜜蜂中肠内孢子载量达到最高,随后出现蜂群衰竭症状。2009–2010 年间,Traver 等<sup>[40]</sup>对弗吉尼亚州西南部蜂群内采集蜂的感染率和孢子载量结合 qPCR 的方法进行分析,结果表明东方蜜蜂微孢子虫的感染在春末夏初达到顶峰,其余时间有所下降,这与 Gisder 等<sup>[41]</sup>在德国的实验结果相似,导致这种差异的原因可能与取样时间、测量指标、当地环境和气候有关。显然,东方蜜蜂微孢子虫的发病特点是复杂的,评估感染水平的季节性变化需要从多个方面进行综合考量。

### 3 临床症状

东方蜜蜂微孢子虫在感染蜂群后,主要经历无症状阶段、取代阶段、假恢复阶段和蜂群衰竭四个阶段<sup>[42]</sup>。无症状阶段主要在感染的第一个春季到秋季,蜂群感染后外观不明显,低于 60% 的采集蜂被感染,单只蜜蜂中肠内东方蜜蜂微孢子载量低于 100 万个。取代阶段是指由于蜜蜂出房前体内几乎不含有孢子,蜂巢内工蜂可以培育较多幼虫,使群势重新恢复。假恢复阶段一般发生在第二年春季到秋季,由于外界蜜源充足,蜂群略有恢复,但整体上感染开始向巢内蜂移动。最后是蜂群衰竭阶段,蜂群内蜜蜂数量突然下降到 60% 左右,采集蜂东方蜜蜂微孢子感染率大于 65%,哺育蜂孢子感染率大于 40%,单只蜜蜂中肠内孢子载量大于 100 万个。若发生在寒冷季节,单只蜜蜂中肠内孢子载量可超过 1000 万个,蜂王和幼虫也会因感染东方蜜蜂微孢子虫而死亡<sup>[39]</sup>。

蜜蜂个体在感染东方蜜蜂微孢子虫后,免疫防御、新陈代谢和营养消化功能受到扰乱。东方蜜蜂微孢子虫会导致被感染蜜蜂免疫受到抑制,

抗菌肽 *Abaecin*、*Apidaecin*、*Hymenoptaecin* 和 *Defensin1* 的基因表达水平显著降低<sup>[43–44]</sup>,由于其发病过程缓慢而漫长,随时间推移逐渐显现下痢、腹部膨大、中肠显白色等明显症状。东方蜜蜂微孢子虫能够诱导中肠和大脑中与营养代谢和激素分泌相关的基因表达的变化<sup>[45–47]</sup>。孢子从蜜蜂中肠中获得能量,损害蜜蜂上皮细胞,并随着感染的时间在数量上不断增加,这增加了蜜蜂的氧化应激<sup>[18,45–46]</sup>,同时咽下腺等与营养敏感相关的结构和功能发生变化<sup>[48–51]</sup>。在中肠蛋白质组中,与能量和蛋白质的代谢、抗氧化防御有关的结构也发生了改变<sup>[52–53]</sup>。因此受感染蜜蜂表现出明显的饥饿感和对蔗糖敏感度的增加,并且不愿意与其他个体分享食物<sup>[17,19]</sup>。同时被感染蜜蜂会出现早熟觅食、恶劣天气条件下外出觅食和盗蜂现象,这些非正常的觅食行为不仅降低了蜜蜂的采集能力,更会使大量蜜蜂无法返回蜂箱,蜂群整体年龄结构发生改变,并可能进一步导致蜂群的损失<sup>[16,39,54–58]</sup>。

### 4 病理变化

东方蜜蜂微孢子虫只在细胞质中发育繁殖而不入侵细胞核。正常蜜蜂中肠上皮细胞的细胞核呈规则的圆球状,而感染东方蜜蜂微孢子虫后,中肠细胞的细胞膜完全被破坏,细胞间界限不明显;细胞质中存在大量处于不同发育阶段的孢子;随后细胞核开始膨大,并因成熟孢子的挤压而呈现不规则形状,细胞核内染色质凝集,核膜略有消融;正常中肠细胞中的线粒体呈长椭圆形,板层状嵴突与线粒体长轴垂直,感染东方蜜蜂微孢子虫后,线粒体变小,嵴增宽且排列方向改变,有些线粒体嵴减小甚至消融。正常中肠细

胞中的粗面内质网排列整齐，多呈密集平行排列的扁平囊状，核糖体附着在膜外表面，感染东方蜜蜂微孢子虫后，粗面内质网散乱于细胞质中，排列紊乱，甚至被挤压成碎片直至解体<sup>[29]</sup>。利用传统的 HE 对蜜蜂中肠切片进行染色，发现被感染的中肠上皮细胞胞浆扩张，胞核颜色鲜艳，顶端移位。细胞腔内常残留充满成熟孢子的分泌球和游离成熟孢子。此外，周围经常出现断裂的细胞膜。某些粪便小球内含有许多孢子，周围环绕着营养物质和花粉<sup>[59]</sup>，在回肠内未见明显病变。

## 5 诊断

通过流行病学和特征性临床症状，可以作出初步诊断，确诊需要通过形态学检测、免疫学检测和分子生物学诊断，主要包括东方蜜蜂微孢子虫的分离鉴定、涂片镜检、单克隆抗体制备、PCR 检测等。并注意与蜜蜂微孢子病的鉴别诊断。

### 5.1 形态学检测

随机抓取 20 只疑似患有东方蜜蜂微孢子

虫的蜜蜂，解剖其中肠于 1.5 mL EP 管中，加入 500  $\mu$ L 双蒸水，利用研磨棒进行研磨。取悬浮液 20  $\mu$ L 于载玻片，盖上盖玻片，在 400 倍光学显微镜下进行镜检(图 3)。若观察到有椭圆形，具有折光性的个体，即可确诊微孢子的存在。

### 5.2 免疫学检测

通过对东方蜜蜂微孢子虫特定的 SWP-32 抗体进行酶联免疫吸附实验 (Enzyme-linked immunosorbent assay, ELISA)，可以确定目的蛋白或者激素的存在，并通过分光光度计和标准样对检测样品的孢子含量和感染程度进行定性<sup>[60]</sup>。

### 5.3 分子生物学诊断

东方蜜蜂微孢子虫具有较厚的孢子壁，需要采用玻璃珠破碎法或液氮研磨法提取模板 DNA，其基因组全长约 7.86 Mb<sup>[61]</sup>，可用于多重 PCR。通过正向引物 (5'-CGGCGACGATGTGATATGAA AATATTAA-3') 和反向引物 (5'-CCCGGTCATTC TCAAACAAAA-AACCG-3') 进行扩增。PCR 反应

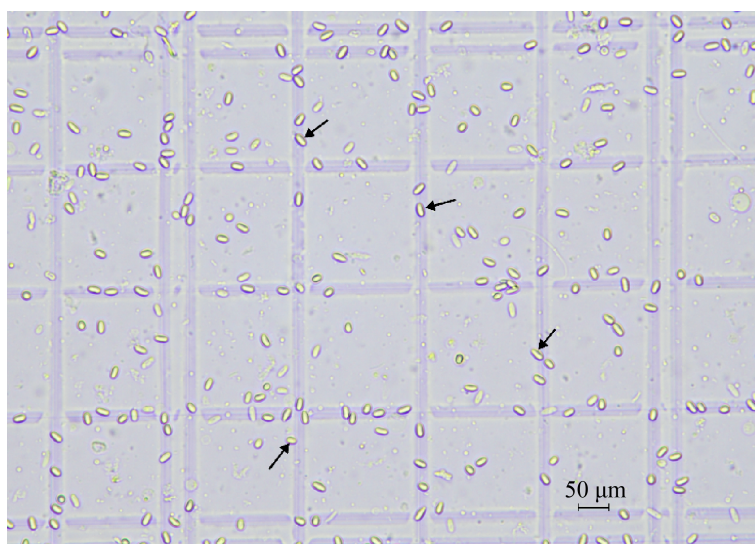


图 3. 400 倍光学显微镜下东方蜜蜂微孢子虫

Figure 3. *Nosema ceranae* under light microscope (400 times).



程序为 94 °C 10 s, 62 °C 15 s, 72 °C 30 s, 25 个循环, 每个连续循环加 2 s 的延伸循环, 最后在 72 °C 下延伸 7 min。其最终产物为 218 bp, 通过琼脂糖凝胶电泳可进行检测<sup>[62]</sup>。

## 6 防治方法

在现有研究中, 尚未发现一种高效抑制或杀灭东方蜜蜂微孢子虫、成本低廉和无残留的药物和方法。但在营养防治、基因水平防治、天然植物提取物防治和微生物防治等方面已有许多探索。

### 6.1 抗生素

烟曲霉素早在 1949 年从真菌烟曲霉 (*Aspergillus Fumigatus*) 发酵液中提取出来, 也是目前在美国唯一经过药品注册和认证的对微孢子感染有效的药物<sup>[63]</sup>。烟曲霉素在美国养蜂业中被应用于控制蜜蜂微孢子虫已有 50 多年历史, 3% 的烟曲霉素对东方蜜蜂微孢子虫也具有抑制作用<sup>[64-65]</sup>。烟曲霉素通过抑制东方蜜蜂微孢子虫蛋氨酸氨基肽酶 2 (*MetAP2*) 的表达, 干扰东方蜜蜂微孢子虫行使正常细胞功能所必需的蛋白质修饰来发挥作用<sup>[66]</sup>。但最新研究表明, 烟曲霉素具有相当强烈的毒性, 会导致蜜蜂染色体畸变和下咽腺超微结构的改变<sup>[63]</sup>, 因此烟曲霉素在美洲以外的许多国家被禁止使用。

### 6.2 肠道微生物对微孢子的感染和预防具有积极意义

随着测序技术的逐渐成熟, 测序的深度在不断提高, 而测序成本却大幅下降<sup>[67]</sup>, 人们对肠道微生物的研究日益增多, 有研究发现, 当病原体侵染宿主时, 微生物群落会产生戏剧性的反应,

通过改善蜜蜂肠道微生态环境, 对提高蜜蜂对病原微生物的抵抗力具有重要作用<sup>[68]</sup>。东方蜜蜂微孢子从侵染宿主细胞到孢子成熟大约需要 4 d 时间<sup>[12]</sup>, 在这一过程中, 东方蜜蜂微孢子虫会不可避免地受到肠道微生物的影响。

在成年蜜蜂身上已经确定了 8 个核心菌群, 包括 *Gilliamella apicola*、*Frischella perrara*、*Snodgrassella alvi*、*Lactobacillus mellis*、*Lactobacillus Firm5*、*Bifidobacterium asteroides*、*Bartonellaceae* 和 *Acetobacteraceae*<sup>[69]</sup>。这 8 个核心菌群以及一些更低频的微生物可能会参与到蜜蜂肠道对花粉和蜂蜜的消化, 宿主并未提供营养<sup>[70]</sup>。研究表明, 当肠道微生物受到抗生素干扰时, 蜜蜂更容易受到东方蜜蜂微孢子虫及其他病原体的感染<sup>[71-72]</sup>。*Frischella perrara* 会触发蜜蜂的黑化反应, 刺激宿主产生具有细胞毒性的醌类中间产物, 醌类中间产物会聚合成黑色素沉积到入侵的病原体周围, 起到隔离杀死病原体的作用<sup>[73]</sup>。乳酸菌作为饲料添加剂对东方蜜蜂微孢子的感染具有抑制作用<sup>[74]</sup>。Szymaś 等<sup>[75]</sup>在人工合成花粉中添加 Biogen 及 Trilac 两种益生菌制剂提高了蜜蜂中肠内围食膜的存在。而围食膜具有保护中肠上皮细胞和有助于食物消化吸收的功能, 从而提高了蜜蜂肠道的抵抗力。Rubanov 等<sup>[76]</sup>对感染东方微孢子与未感染微孢子蜜蜂体内肠道菌群进行比对, 发现两个特定的 *Gilliamella* 菌株与东方蜜蜂微孢子虫的感染强度呈正相关。但也有研究发现, 共生菌 *Gilliamella Apicola* 和 *Snogrsella Alvi* 具有提高宿主对食物消化能力和病原体防御的功能, 这可能与不同菌株之间的序列差异导致的功能变异有关<sup>[77-78]</sup>。

显然, 蜜蜂和肠道微生物会对东方蜜蜂微孢

子虫的感染作出反应, 这为东方蜜蜂微孢子虫的防治提供了一种十分重要的非化学手段<sup>[79]</sup>, 但不适当的益生菌制剂补充也可能导致肠道微生物的失调和对病原微生物易感性的增加<sup>[80]</sup>, 因此利用益生菌防治东方蜜蜂微孢子虫感染仍有巨大的研究空间和价值。

### 6.3 天然提取物对微孢子的抑制作用

天然提取物对东方蜜蜂微孢子虫的抑制作用也同样受到人们的广泛研究。各种天然提取物已被证明在饲喂蜜蜂治疗东方蜜蜂微孢子虫感染后, 可以提高蜜蜂的存活率和降低孢子的载量<sup>[81-85]</sup>。蜂胶是工蜂通过采集树脂, 并混入上颚腺和蜡腺分泌物形成的产物<sup>[86]</sup>, 是蜜蜂建造巢房的主要物质, 也是在传统中医药中重要的材料。蜂胶提取物在抗菌抑菌<sup>[87]</sup>、解毒、杀灭病原微生物<sup>[88]</sup>等方面的功效已被证实。Alessandra 等<sup>[89]</sup>试验证明蜂胶对东方蜜蜂微孢子虫的感染具有抑制作用, 但高浓度的蜂胶对蜜蜂存在显著的致死作用, 这可能与蜂胶内所含有的一些多酚类物质有关。莱菔硫烷、香芹酚、柚皮素、1%浓度的牛膝提取液被证明对东方蜜蜂微孢子虫的感染和发育具有抑制作用, 但与蜂胶一样存在对蜜蜂致死的作用<sup>[83,90]</sup>。

从蜂胶和植物中所获得的粗提取物化学成分往往是复杂且未知的, 提取物成分的降解及变质等会影响天然提取物对东方蜜蜂微孢子的防治效果。利用天然提取物来防治东方蜜蜂微孢子的生产过程必须高度标准化, 才能保证治疗效果<sup>[91]</sup>。虽然天然植物提取物还存在一定的缺陷, 但从药物残留和耐药性方面考虑, 利用植物提取物治疗或预防东方蜜蜂微孢子虫的感染仍是当下研究的一大热点。

### 6.4 RNA 干扰技术对微孢子的防治效果

RNA 干扰(RNAi)是一种由双链 RNA (dsRNA)引起的转录后基因沉默的自然机制, 双链 RNA 在序列上与沉默的基因同源, 已被用于操纵几种生物中的基因表达<sup>[92]</sup>。利用 RNA 干扰技术来进行基因敲除的方法已被广泛用于控制害虫数量、控制昆虫病原性疾病和研究特定基因在许多昆虫物种中的功能作用<sup>[93-95]</sup>。对意大利蜜蜂的完整基因组分析揭示了该物种中存在与 RNA 沉默有关的遗传机制。东方蜜蜂微孢子虫和蜜蜂微孢子虫基因组的可获得性以及对这 2 个微孢子虫物种的比较基因组分析<sup>[96]</sup>, 为蜜蜂宿主细胞内寄生虫的粘附、入侵、免疫逃避、定殖和复制有关的毒力因子的鉴定提供了条件。对东方蜜蜂微孢子虫在感染过程中的生活史和致病性了解的提高, 使我们能够利用 RNA 干扰技术来治疗蜜蜂微孢子病。现有研究表明, 沉默微孢子虫毒力因子和宿主免疫抑制基因可以减少寄生虫载量, 激活蜜蜂免疫反应, 并改善受感染蜜蜂的整体健康<sup>[97-98]</sup>。

极管是微孢子穿透宿主细胞膜, 将具有感染性孢子质传递到宿主细胞内, 完成对宿主侵袭感染的重要结构。富含脯氨酸的极管蛋白 1、富含赖氨酸的极管蛋白 2 和分子量大于 135 kDa 的极管蛋白 3 是已知的组成孢子极管的主要蛋白。Rodríguez-García 等以蜜蜂微孢子同源、分子量大于 13 kDa 的极管蛋白 3 为靶基因, 饲喂极管蛋白 3 区域所对应的 dsRNA, 可以使感染东方蜜蜂微孢子虫的蜜蜂在处理后的第 10 天, 极管蛋白 3 表达下调, 蜜蜂体内微孢子虫的载量明显降低, 与免疫相关的 *Apidaecin*、*Abaecin*、*Hymenoptaecin* 和 *defensin-1* 4 个基因表达发生上调<sup>[97,99]</sup>。

*Dicer* 基因对细胞核酸内切酶具有广泛的调控作用, Huang 等<sup>[100]</sup>利用小 RNA 干扰技术, 通过抑制微孢子 *Dicer* 基因的表达, 降低了 70% 微孢子的数量, 与细胞增殖相关基因受到抑制, 说明微孢子繁殖受到抑制。同时, *mucin-2-like* 在 *Dicer* 基因受到抑制后显著上调, 增强了中肠黏膜上皮细胞对微孢子的抵抗力。结果表明 *Dicer* 可调控东方蜜蜂微孢子虫的繁殖。

通过抑制宿主免疫抑制基因表达、提高蜜蜂抵抗力也是治疗和预防东方蜜蜂微孢子虫感染的重要手段。研究表明, Wnt 通路对昆虫 Toll 通路上免疫基因的表达具有抑制作用<sup>[101]</sup>, 裸露角质层基因(*naked cuticle*, *nkd*)是 Wnt 通路上的重要一员<sup>[102]</sup>。Li 等<sup>[103]</sup>通过沉默蜜蜂 *nkd* 基因, 上调了 *Apidaecin*、*Abaecin*、*Hymenoptaecin* 和 *defensin-1* 4 个免疫基因的表达, 进而降低了宿主体内东方蜜蜂微孢子虫的载量, 延长了蜜蜂寿命。这一结果清晰地表明, 沉默宿主 *nkd* 基因的表达可以激活宿主的免疫反应, 抑制东方蜜蜂微孢子虫的繁殖, 改善蜜蜂的整体健康状况。

## 6.5 抗东方蜜蜂微孢子虫蜂王的培育

实践证明, 选择性繁殖、更换蜂王等蜂群管理手段对蜂群的多发性疾病具有很好的预防和缓解作用。通过人工选育的方法, 培育出对微孢子感染具有抗性或耐性的蜂种, 所产生的意义是长远和重大的。这不仅减少了蜂农在时间和金钱方面的投入, 更减少了耐药性和药物残留发生的可能性。在丹麦, 蜂农通过 20 多年的选育, 已经培育出一种对东方蜜蜂微孢子虫具有抗性的蜂种<sup>[104]</sup>。在俄罗斯<sup>[105]</sup>、乌拉圭<sup>[106]</sup>也有报道发现对东方蜜蜂微孢子虫具有天然抗性的蜂种。这表明, 在蜜蜂群体中足够的遗传变异可以产生对东

方蜜蜂微孢子虫具有抗性的品种。

通过以上研究可知, 通过药物防治东方蜜蜂微孢子虫其效果是相对有限的, 且目前尚无法律允许的药物用于养蜂业的生产。现阶段天然植物提取物、蜂胶提取并没有实现批量生产, 且存在使用浓度与蜜蜂致死率的矛盾尚待解决。对于蜜蜂肠道微生物和营养状况的研究, 证实了提高蜜蜂自身抵抗力对抑制东方蜜蜂微孢子虫的积极意义。RNA 干扰技术则存在着成本高和临床应用繁琐的缺点。在生产实践中加强蜂群的饲养管理, 选择地势较高、阳光充足和较为安静的环境, 饲喂干净和优质的蜂粮, 提高蜂群自身抵抗力是防治东方蜜蜂微孢子虫的有效途径。

## 7 展望

在东方蜜蜂微孢子虫首次发现的 10 年后, 就有研究表明在美国和欧洲先后暴发的蜜蜂崩溃综合症(colony collapse disorder, CCD)与东方蜜蜂微孢子虫存在间接联系<sup>[39]</sup>。2013 年, 张建燕等<sup>[107]</sup>对我国 16 个主要养蜂地区的蜜蜂微孢子感染情况进行了调查和检测, 在 68 份检测样品中, 东方蜜蜂微孢子虫在西方蜜蜂的体内感染率高达 98.5%。东方蜜蜂微孢子虫在我国以及世界范围内已广泛流行, 但在现有阶段, 鲜有资料就东方蜜蜂微孢子虫传染病学特点进行总结和报道。

东方蜜蜂微孢子虫病的发生和流行是由传染源、传播途径和易感动物相互联系、相互作用引起的复杂过程, 从传染病学角度做好东方蜜蜂微孢子虫病的防治工作具有重要意义。在综合防治东方蜜蜂微孢子虫病时, 需要果断采取消灭传染源、切断传播途径和保护易感动物的措施, 以此阻止传染病流行发展过程中 3 个必要因素之间



的相互联系。在现阶段,人们已从病原学特点、临床特征、诊断及分子生物学水平对东方蜜蜂微孢子虫病进行了解,在治疗手段方面经历了从单纯的利用抗生素治疗到利用肠道微生物、天然提取物、RNA 干扰及育种技术对蜜蜂进行预防 and 治疗的探索,但在切断东方蜜蜂微孢子虫的传播途径方面仍然需要进行大量的探索和研究。

通过确定东方蜜蜂微孢子虫的感染特征以及开发出一套预防和治疗东方蜜蜂微孢子虫病的治疗方法意义重大。对东方蜜蜂微孢子虫的治疗和控制不仅可以降低蜂群的损失,更能使其他野生授粉者受益<sup>[108–110]</sup>。蜜蜂与东方蜜蜂微孢子虫之间的研究也可能为人类微孢子虫病的研究和治疗提供借鉴和模型<sup>[111]</sup>,通过对蜜蜂病理学的深入研究为人类的医疗实践提供重要的参考。

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# The epidemiological analysis of *Nosema ceranae*

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**Abstract:** Nosemosis is an infectious disease caused by *Nosema ceranae*, which infects honey bees globally. *Nosema ceranae* infection led to premature aging, decreased nursing and productivity, as well as fecundity. Under extreme circumstances, the infection caused colony collapses. In this study, we summarized the etiology, epidemiology and the control methods of *Nosema ceranae* in the last ten years. This study aims to improve the understanding of *Nosema ceranae* biology and provide new insights for the parasite control.

**Keywords:** *Nosema ceranae*, epidemiological analysis, prevention and control methods, honeybee

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