



汤新明, 梁琳, 梁瑞英, 于咏兰, 丁家波, 刘贤勇, 索勋. 真核载体疫苗的创新性设计与优化: 以艾美耳球虫为例[J]. 中国农业大学学报, 2025, 30(09):132-140.
TANG Xinming, LIANG Lin, LIANG Ruiying, YU Yonglan, DING Jiabo, LIU Xianrong, SUO Xun. Optimization and novel design strategies for eukaryotic vector vaccines: Insights from *Eimeria* research[J]. *Journal of China Agricultural University*, 2025, 30(09):132-140.
DOI: 10.11841/j.issn.1007-4333.2025.09.12

真核载体疫苗的创新性设计与优化: 以艾美耳球虫为例

汤新明¹ 梁琳¹ 梁瑞英¹ 于咏兰² 丁家波¹ 刘贤勇² 索勋²

- (1. 中国农业科学院 北京畜牧兽医研究所/农业农村部动物生物安全风险预警及防控重点实验室(北方)/农业农村部
兽用生物制品与化学药品重点实验室, 北京 100193;
2. 中国农业大学 动物医学院/兽医公共卫生安全全国重点实验室, 北京 100193)

摘要 目前, 病毒载体疫苗已在人用和兽用领域实现商业化应用, 而细菌及寄生虫载体疫苗虽在基础研究方面取得进展, 却尚未突破产业化瓶颈, 其核心问题在于载体系统的设计与优化亟待理论创新和技术突破。本综述聚焦我国学者原创性提出的艾美耳球虫疫苗载体系统, 系统阐释寄生虫等真核载体的分子设计原理、优化策略及免疫增效机制。结果表明: 1) 真核载体疫苗的效能提升可概括为“一体两翼”工程: 艾美耳球虫独特的生物学和免疫学特性是其作为真核载体疫苗研发的基础, 是“一体两翼”的主体。2) 艾美耳球虫载体自身和异源抗原表达时空的设计与优化构成了载体疫苗效能提升的“两翼”。3) 载体-抗原协同优化是显著增强真核载体疫苗激发宿主保护性免疫应答强度的集成。综上, 艾美耳球虫作为真核疫苗载体具有可行性, 随着基因编辑技术、合成生物学、计算疫苗学和递送技术的融合发展, 艾美耳球虫真核载体疫苗系统有望突破实验室研究阶段, 在畜禽疫病防控等领域实现转化应用, 也为其他真核载体疫苗的研发提供参考。

关键词 载体疫苗; 艾美耳球虫; 设计与优化

中图分类号 S855.9

文章编号 1007-4333(2025)09-0132-09

文献标志码 A

Optimization and novel design strategies for eukaryotic vector vaccines: Insights from *Eimeria* research

TANG Xinming¹, LIANG Lin¹, LIANG Ruiying¹, YU Yonglan², DING Jiabo¹,
LIU Xianrong², SUO Xun²

(1. Institute of Animal Science/Key Laboratory of Animal Biosafety Risk Prevention and Control (North) of MARA/ Key Laboratory of Veterinary Biological Products and Chemical Drugs of MARA, Chinese Academy of Agricultural Sciences, Beijing 100193, China;

2. College of Veterinary Medicine/State Key Laboratory of Veterinary Public Health Security, China Agricultural University, Beijing 100193, China)

Abstract Currently, viral vector vaccines have achieved commercial success in both human and veterinary medicine, while bacterial and parasitic vector vaccines, despite progress in basic research, have yet to overcome industrialization bottlenecks. The core challenge lies in the urgent need for theoretical innovation and technological breakthroughs in vector system design and optimization. This study focuses on the *Eimeria*-based vaccine vector system, originally proposed by Chinese researchers, and systematically elucidates the molecular design principles, optimization strategies, and immune-enhancing mechanisms of eukaryotic parasitic vectors. Key findings include: 1) The efficacy enhancement of eukaryotic vector vaccines can be summarized as the “One

收稿日期: 2025-05-27

基金项目: 国家重点研发计划项目(2023YFD1802400); 中国农业科学院青年创新专项(Y2023QC10)

第一作者: 汤新明(ORCID: 0000-0003-0127-1235), 副研究员, 主要从事动物重要寄生虫病新型疫苗研发, E-mail: tangxinming@caas.cn

Body, Two Wings” project. The unique biological and immunological characteristics of *Eimeria* parasites serve as the foundation for its development as a eukaryotic vector vaccine, forming the “main body” of the project. 2) The design and optimization of both the *Eimeria* vector itself and heterologous antigens expression pattern, guided by cutting-edge theories and technologies, constitute the “two wings” for enhancing the efficacy of vector vaccines. 3) Vector-antigen synergistic optimization is an integrated approach that significantly strengthen the host’s protective immune response elicited by eukaryotic vector vaccines. In conclusion, *Eimeria* demonstrates feasibility as a eukaryotic vaccine vector. With the integration of genetic editing, synthetic biology, computational vaccinology, and delivery technologies, the *Eimeria* vector system holds promise for translational applications in livestock disease prevention and control, while also serving as a reference for the development of other eukaryotic vector vaccines.

Keywords vector vaccine; *Eimeria*; design and optimization

疫苗接种长期以来被视为最有效的公共卫生干预措施之一,在显著降低人类和动物传染病的发病率和死亡率方面发挥了关键作用。传统疫苗平台,如减毒活疫苗和灭活疫苗,虽已被广泛应用并取得显著成效,但在应对复杂病原体、新发传染病威胁以及不断变化的抗原谱方面,局限性日益显现。这些挑战促使研究者积极探索下一代疫苗策略,其中活载体疫苗的发展备受关注^[1-2]。活载体疫苗通过微生物(如病毒、细菌、真菌或原生动物)作为递送抗原的工具,利用其生物学机制诱导宿主产生强大且持久的免疫应答^[1,3-4]。随着基因工程技术的不断进步以及对宿主-病原相互作用认识的加深,多种活载体疫苗已从试验研究阶段逐步转化为商业化产品,展现出良好的应用前景。

在众多潜在的原生动物载体中,艾美耳球虫(*Eimeria* spp.)因其在兽医领域中的广泛影响而备受关注。艾美耳球虫是畜禽球虫病的主要病原,造成了巨大经济损失^[5]。基于活卵囊的鸡球虫病疫苗是动物传染病防控中最为成功的疫苗之一。无论是野毒株还是减毒株的活卵囊疫苗,都能在接种鸡体内诱导持久的保护性免疫应答^[6-7]。艾美耳球虫的免疫学特性加之其独特的生物学特性优势使其成为理想的疫苗载体候选,但这需要对疫苗载体和抗原表达进行设计和优化,即艾美耳球虫疫苗载体开发的“一体两翼”工程:一体即艾美耳球虫的特性是疫苗载体开发的基础;两翼则是分别从载体和抗原2个方面持续优化,以实现抗原运载性能和免疫保护效果的提升(图1)。本综述围绕艾美耳球虫疫苗载体科学设想的提出、转染和筛选平台的构建、抗原的表达与免疫效能的提升等多维度系统综述载体疫苗创新性设计和优化策略,助力艾美耳球虫载体疫苗的研发,为其他载体疫苗设计和优化提供

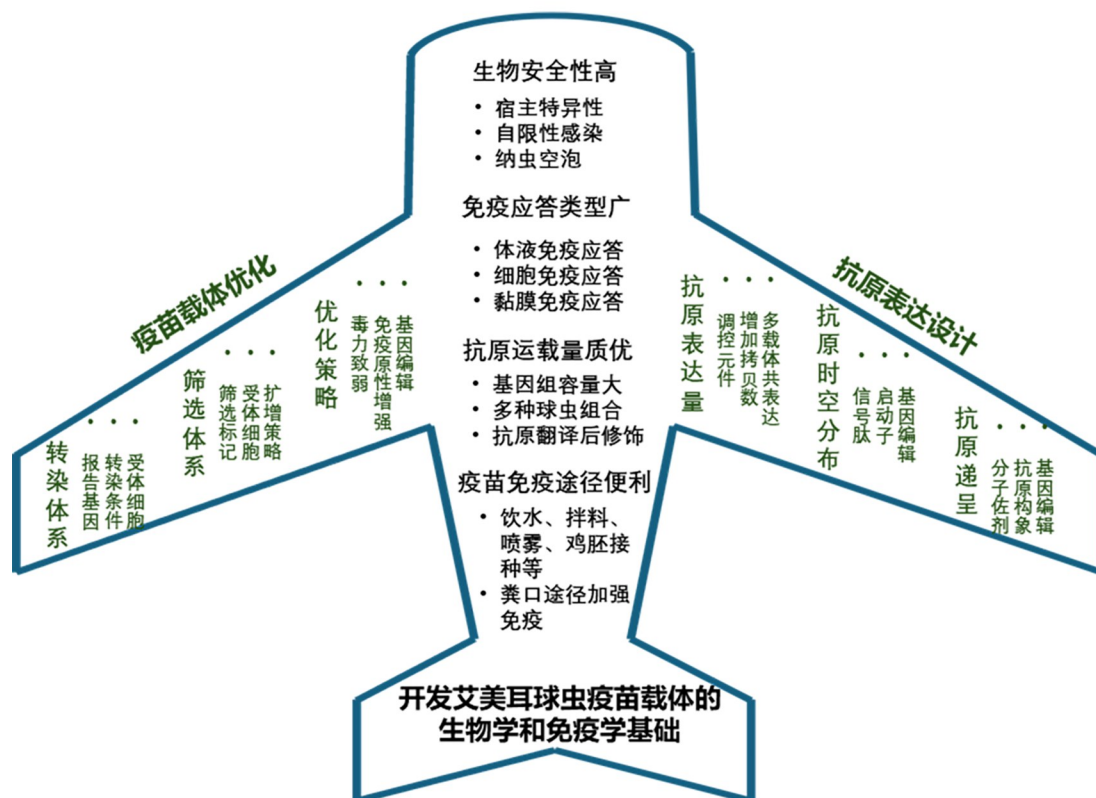
参考。

1 疫苗载体优化:遗传操作平台的建立与优化

1.1 疫苗载体开发的前提:转染系统

顶复门寄生虫如弓形虫(*Toxoplasma gondii*)和疟原虫(*Plasmodium* spp.)的转染系统早在20世纪90年代初建立^[17-18],但艾美耳球虫在体外难以完成生活史以及缺乏生物信息学工具,导致其遗传操作研究相对滞后。1998年,Kelleher和Tomley使用 β -半乳糖苷酶(β -gal)作为报告酶,首次报道了艾美耳球虫的遗传操作研究^[19]。研究表明,艾美耳球虫的子孢子阶段可以实现质粒介导的转染,并可在*EtMic-1*启动子的驱动下成功表达外源*lacZ*基因^[19]。由于X-Gal检测系统灵敏度和应用场景有限,艾美耳球虫的转染研究此后停滞了近十年。直到2007年引入荧光蛋白作为报告基因,该领域的研究才得以迅速发展^[20]。

电穿孔是将外源DNA或RNA引入细胞内最有效的方法之一。利用电穿孔技术可将环状或线性质粒,PCR产物转染至艾美耳球虫子孢子或裂殖子^[21-22]。早期的瞬时转染研究使用了完全Cytomix缓冲体系和环形质粒电穿孔系统,但转染效率极低^[19,23]。当采用线性化质粒并加入限制性内切酶进行转染后,效率提升了约200倍,这一方法被称为限制性内切酶介导的整合(Restriction enzyme-mediated integration, REMI)^[22]。利用REMI技术结合AMAXA核转染系统,可在鸡、兔、小鼠或大鼠艾美耳球虫子孢子或裂殖子中实现极高的转染效率^[21,24-26]。在体外培养于鸡肾原代细胞或牛肾细胞系后,通过荧光显微镜即可直观观察转染孢子中荧光报告蛋白的表达并统计转染效率。因此,艾美耳球虫的瞬时转染系统不仅为筛选



一体是指艾美耳球虫作为疫苗载体的生物学和免疫学基础,即疫苗载体的优势,包括:1)艾美耳球虫的生物安全性高,体现在艾美耳球虫具有严格的宿主特异性,其感染为自限性感染,且在寄生的细胞内形成纳虫空泡结构,生物安全性高^[8-9];2)艾美耳球虫是高度免疫原性的寄生虫,能诱导广泛的免疫应答,包括体液免疫应答、细胞免疫应答和黏膜免疫应答等^[10];3)艾美耳球虫为真核生物,基因组较大,约为55~60 Mbp,编码约8 000~9 000个基因,具备较强的异源抗原基因携带能力^[11-12],还可对表达的抗原进行翻译后修饰来维持异源抗原的天然构象和免疫原性^[13];4)基于艾美耳球虫载体的重组疫苗可经口免疫(如饮水、拌料、喷雾等)或鸡胚接种,省时省力^[14];并通过粪-口途径反复免疫实现异源抗原特异性免疫应答的自动加强和提升^[15-16]。两翼是指重组艾美耳球虫疫苗载体设计与优化时分别从载体和抗原两方面并重,包括转染和筛选体系的优化、疫苗载体性能的优化、抗原表达的调控与设计、增强抗原免疫原性的新策略等。

“One Body” refers to the biological and immunological foundation of *Eimeria* as a vaccine vector, i. e., the advantages of the vaccine vector, including: 1) High biosafety: *Eimeria* exhibits strict host specificity, causes self-limiting infections, and forms parasitophorous vacuoles within host cells, ensuring high biosafety^[8-9]; 2) Strong immunogenicity: *Eimeria* is a highly immunogenic parasite capable of inducing comprehensive immune responses, encompassing humoral immunity, cellular immunity, and mucosal immunity^[10]; 3) High genomic carrying capacity: As a eukaryote, *Eimeria* has a relatively large genome (55-60 Mbp), encoding approximately 8 000-9 000 genes, providing substantial capacity for carrying heterologous antigen genes^[11-12]. It also enables post-translational modifications to maintain the native conformation and immunogenicity of expressed antigens^[13]; 4) Convenient immunization: Recombinant *Eimeria*-based vaccines can be administered orally (e. g., via drinking water, feed mixing, or spray) or through *in ovo* inoculation, saving time and effort^[14]. Additionally, the fecal-oral route allows repeated immunization, enabling auto-boosting and enhancement of heterologous antigen-specific immune responses^[15-16]. “Two Wings” refers to the dual emphasis on vector optimization and antigen design when engineering recombinant *Eimeria* vaccine vectors, including: Optimization of transfection and selection systems, enhancement of vaccine vector performance, regulation and design of antigen expression pattern, and novel strategies to improve antigen immunogenicity.

图1 艾美耳球虫疫苗载体开发的“一体两翼”设计与优化

Fig. 1 The “One Body, Two Wings” theory of design and optimization for *Eimeria*-based vaccine vector development

和优化启动子、信号序列及其他调控元件提供了高效平台,也为构建稳定表达的转基因球虫虫株奠定了基础^[27-28]。

1.2 疫苗载体开发的基础:筛选系统

转染的子孢子(如柔嫩艾美耳球虫、和缓艾美耳球虫和堆型艾美耳球虫)或裂殖子(如毒害艾美耳球

虫)可根据艾美耳球虫的寄生部位,通过泄殖腔接种或经静脉注射接种至雏鸡体内^[21, 23, 29-30]。对于免艾美耳球虫,也可以通过手术方式将转染的子孢子接种至肠腔中,这是一种高效获得转基因寄生虫的方法^[25, 31]。通过体内多代选择,并结合流式细胞分选(Fluorescence-activated cell sorting, FACS)技术,可获得表达荧光报

告蛋白的重组艾美耳球虫卵囊^[32-33]。但受到多种因素的制约,仅靠 FACS 筛选效率较低。为提高筛选效率,研究引入了利用突变弓形虫二氢叶酸还原酶-胸苷酸合成酶(Dihydrofolate reductase-thymidylate synthase, DHFR-TSm2m3, TgDHFR)构建的乙胺嘧啶抗性筛选系统,已成功用于高效筛选重组疟原虫和弓形虫^[34],该系统与 FACS 联合使用,显著提升了重组艾美耳球虫的筛选效率^[31-32]。药物抗性基因与荧光蛋白基因可以进行多种策略的配比或联合使用来提升重组艾美耳球虫的筛选效率。同时也是艾美耳球虫连续或多次进行遗传操作实现多基因表达的有效策略。利用实验室诱导和进化筛选的策略已经成功筛选出常山酮的耐药位点,基于耐药位点抗体系统也正在艾美耳球虫的遗传操作筛选中应用。常用抗球虫药物达数十种,抗药基因的应用弥补了常规用于流式分选的荧光报告基因选择不足的弊端,将药物抗性筛选基因与荧光报告基因有望极大提升遗传操作的效率和拓展艾美耳球虫遗传操作的范围^[35-36]。随着测序技术成本降低,艾美耳球虫基因编辑技术的不断完善,越来越多的抗球虫药物耐药位点被逐步解析和验证,可为艾美耳球虫的遗传操作提供更多筛选标记,提升筛选效率^[37-39]。外源基因的整合位点或精准定位载体疫苗设计核心要素之一。

经过多代筛选后,重组艾美耳球虫能稳定表达外源基因,外源质粒可成功整合至寄生虫基因组中,但不同个体的整合位点有所差异^[29, 40]。在采用药物和 FACS 双重选择压力获得的重组艾美耳球虫群体中,仅检测到一个整合位点,提示经过持续筛选后,该群体趋于稳定^[41]。在单个卵囊克隆子代中也检测到唯一整合位点,并且即便在没有选择压力的条件下,报告基因仍能稳定表达^[32]。Southern blot 杂交结果亦显示,在单一孢子囊克隆的基因组中仅检测到一份外源 DNA 拷贝^[42]。不同重组艾美耳球虫系统中外源质粒在基因组内整合位点的差异,可能导致其繁殖能力存在显著差异。例如,重组艾美耳球虫 TE1 株的卵囊排出量明显降低^[43],而另一株 EtM2e 的卵囊排出量则较其亲本株提高了约 2 倍^[42]。

1.3 疫苗载体优化的核心技术:基因编辑系统

CRISPR/Cas9 系统是一种强大的基因编辑工具,已广泛应用于包括弓形虫、疟原虫、隐孢子虫和利什曼原虫等顶复门寄生虫在内的多种物种。该技术同样适用于艾美耳球虫基因编辑:1)利用柔嫩

艾美耳球虫组蛋白 4(Histone protein 4, EtHis4)启动子和核定位序列可调控化脓链球菌 Cas9 (*Streptococcus pyogenes* Cas9, SpCas9)核酸酶在孢子细胞核中的表达,是 SpCas9 切割寄生虫核基因组的空间基础;2)柔嫩艾美耳球虫具有 2 个 U6 启动子,均能有效调控 sgRNA 表达且表达的 sgRNA 具有生物学活性;3)该系统能介导艾美耳球虫基因组靶位点产生双链断裂(Double-strand breaks, DSBs);4)艾美耳球虫基因组 DSBs 在存在同源片段时可发生同源重组修复^[44]。后续研究者主要从 2 种途径提升基因编辑效率:一是构建稳定表达 SpCas9 的转基因艾美耳球虫株系,以其为亲本进行基因编辑以降低多质粒共转染效率低的影响^[45];二是采用核糖核蛋白(Ribonucleoprotein, RNP)复合物介导的基因编辑,将 FnCas12a 蛋白与向导 RNA 体外孵育形成核 RNP 后进行转染实现基因编辑^[46]。这些基因编辑系统均已成功应用,不同方法各具优劣,适用于不同场景。

2 抗原表达设计:表达调控与免疫效果提升策略

2.1 异源抗原表达时空模式的调控及策略

实现外源基因的稳定表达需要合适的 5' 和 3' 侧翼序列,这些序列可来源于艾美耳球虫或与其进化关系较近的弓形虫基因^[47]。由于艾美耳球虫和弓形虫具有单顺反子转录特性,因此启动子元件通常位于转录起始位点附近,便于识别。目前已有多个来源于艾美耳球虫和弓形虫的启动子被证实适用于驱动报告基因或外源基因的表达^[24, 47-49]。这些启动子具有不同的强度和发育阶段特异性,可根据具体试验目的进行选择^[29, 48]。研究表明,强启动子如 *SAG13* (Surface antigen 13) 或 *EtHis4* 在调控外源抗原表达构建新型载体疫苗时,往往诱导高效的免疫应答^[50-51]。3' 非翻译区(3 terminal untranslated region, 3'-UTR)在转基因生物中调控外源基因表达中也具有重要作用,但调控机制尚不完全清楚。研究证实, *Actin*、*His4*、*Mic2* (Microneme protein 2) 或 *SAG13* 启动子与来源于 *Actin* 基因的 3'-UTR 联合使用时,均能够高效驱动报告基因表达^[28]。

重组艾美耳球虫中外源基因的最终表达模式受到多种因素的共同影响,包括启动子、信号肽序列以及蛋白产物的特性。例如,当将荧光蛋白 EYFP (Enhanced yellow fluorescent protein) 与弓形

虫的致密颗粒抗原8(Dense granule antigen 8, GRA8)的信号肽融合、由 *Actin* 启动子驱动表达时,该蛋白主要被分泌至艾美耳球虫的纳虫空泡^[48, 52];将荧光蛋白融合至艾美耳球虫微线蛋白的C端可实现荧光蛋白在艾美耳球虫的微线体表达,而实现荧光蛋白的膜展示则需要将其插入膜表面抗原基因膜定位序列的上游^[53]。因此,根据抗原基因激发宿主免疫应答的特性选择合适的抗原表达载体设计至关重要。

重组艾美耳球虫表达异源病原体抗原是将其开发为新型疫苗递送载体的首要且关键的一步。重组艾美耳球虫的报告基因或目标基因(Gene of interest, GOI)表达可通过荧光显微观察、间接免疫荧光检测及蛋白印迹(Western blot)等手段检测^[27-28]。使用 His4 或 *Actin* 启动子驱动的报告基因在重组艾美耳球虫生命周期各阶段中持续表达,而若采用 Mic2 或 SAG13 启动子,则在未孢子化卵囊阶段沉默表达^[32, 48]。类似地,在使用相同调控元件的重组艾美耳球虫中,表达巨型艾美耳球虫的免疫标记蛋白1(*EmIMP1*)抗原基因,也可通过 Western blot 和间接免疫荧光方法检测到其表达模式^[50]。在抗体缺少或特异性不佳的情况下,利用质谱分析技术检测异源抗原特异性肽段的表达也是证明抗原基因在重组艾美耳球虫表达的一种新的方法,对其他载体疫苗的设计和开发具有借鉴意义^[54]。

2.2 异源抗原特异性免疫应答水平提升的调控及策略

重组艾美耳球虫表达的异源病原体抗原能够被宿主免疫系统递送并识别,这是将艾美耳球虫开发为疫苗载体的第二个关键步骤。以 EYFP 为模式抗原,在接种重组艾美耳球虫的鸡体内检测到 EYFP 特异性淋巴细胞增殖以及 CD4⁺ 和 CD8⁺ T 细胞中干扰素- γ (IFN- γ)的表达^[55]。与表达定位于细胞质的 EYFP 的重组艾美耳球虫相比,表达定位于微线体的 EYFP 免疫鸡体内产生了更高水平的 EYFP 特异性淋巴细胞增殖反应和更强的 IgA 反应^[55]。这些结果表明,异源抗原在重组艾美耳球虫内的亚细胞定位会影响免疫反应,因此在构建重组疫苗时应予以充分考虑。目前已有多种设计将异源蛋白靶向到重组艾美耳球虫的不同细胞器或孢子腔中^[56-57]。然而,由于缺乏针对特定细胞器(如顶质体、致密颗粒)的抗体,尚难以通过共定位准确

定位外源蛋白的亚细胞定位。例如,将 YFP 与弓形虫的致密颗粒蛋白7(GRA7)融合表达时,在细胞内表达于纳虫空泡,而在胞外则观察到“小颗粒”结构,预测可能定位于致密颗粒中^[49]。但这些“小颗粒”是否为致密颗粒仍有待进一步验证,因为目前缺乏艾美耳球虫致密颗粒的特异抗体和结构学证据。重组艾美耳球虫表达的病毒、细菌或原虫来源的免疫优势或免疫保护性抗原能够被宿主免疫系统识别并诱导免疫应答^[57]。例如,表达传染性法氏囊病病毒(Infectious bursal disease virus, IBDV) VP2 蛋白或传染性喉气管炎病毒(Infectious laryngotracheitis virus, ILTV)糖蛋白I的重组艾美耳球虫可在鸡中诱导产生低滴度的特异性抗体^[54, 58-59]。表达弓形虫表面抗原1(SAG1)的重组柔嫩艾美耳球虫(Et-TgSAG1)在鸡口服免疫一次后,诱导了 TgSAG1 特异性 IgY 抗体产生和 IFN- γ 分泌的外周血单核细胞增殖反应^[51]。有趣的是,Et-TgSAG1 孢子通过腹腔注射免疫小鼠也能有效诱导体液免疫应答,产生以 IgG2a 为主的抗体,该抗血清可识别弓形虫速殖子中的天然 SAG1 抗原^[51]。平行试验表明,表达 *EmIMP1* 的重组柔嫩艾美耳球虫可诱导 *EmIMP1* 特异性抗体产生,且在加强免疫后抗体滴度进一步升高,这些抗体可与天然 *EmIMP1* 抗原发生反应^[50]。此外,接种该重组艾美耳球虫的鸡体内还能检测到巨型艾美耳球虫可溶性蛋白诱导的特异性 IFN- γ 分泌的外周血单核细胞增殖反应^[50]。相比之下,另一种表达免疫保护性抗原—巨型艾美耳球虫顶膜抗原1(*EmAMA1*)的重组艾美耳球虫虽可诱导针对 *EmAMA1* 的抗体反应,但对巨型艾美耳球虫可溶性蛋白提取物中的天然 *EmAMA1* 抗原未表现出反应性^[60]。

将重组艾美耳球虫开发为疫苗载体的第三步是诱导机体对异源病原体感染产生高度免疫保护。研究已对不同重组艾美耳球虫株系在抵御异源病原体感染方面的保护效果进行了评估。表达 *Campylobacter jejuni* 候选疫苗抗原 CjaA 的研究中,SPF (Specific pathogen free)鸡经单次或多次口服免疫后,在后续攻毒感染中相对于未免疫组和接种野生型艾美耳球虫的对照组,表现出 91% 和 86% 的保护率^[61]。表达 TgSAG1 的重组艾美耳球虫接种后也能有效保护鸡只免受弓形虫感染,与未免疫或接种野生型寄生虫的鸡相比,外周免疫器官的炎症程度显著降低。此外,

免疫小鼠在弓形虫速殖子攻毒后生存时间明显延长(从24 h延至96 h)^[51]。另一方面,接种表达 *EmIMP1* 或 *EmAMA1* 的重组艾美耳球虫也能在艾美耳球虫攻虫后显著减少卵囊排出量^[50, 60]。当联合使用2种不同的重组艾美耳球虫进行免疫时,对异源病原体的保护效果进一步增强,为下一代球虫病疫苗的研发开辟了新方向^[62]。

3 重组球虫载体疫苗的优化与提升策略

艾美耳球虫是引起多种畜禽类球虫病的主要病原。尽管由活卵囊构成的疫苗在全球范围内对鸡和火鸡的球虫病防控已取得巨大成功,但关于如何降低其致病性同时保留其免疫原性,以及探索其作为疫苗载体潜力的研究仍在不断进行。

3.1 艾美耳球虫致弱技术提升疫苗载体的安全性

一种传统的疫苗生产方法是对感染鸡体后最早排出的卵囊进行连续传代,这是迄今为止最有效的生产减毒活疫苗株(即所谓“早熟株”)的方法^[63]。该方法在禽类和兔类球虫中均有广泛应用。与野生型艾美耳球虫相比,早熟株的毒力显著降低,但其繁殖能力也明显下降^[64]。另一种传统的减毒疫苗制备方法是将体外释放的子孢子接种至受精鸡胚的尿囊腔中进行连续传代。这一策略通常可以获得毒力较野生型虫株明显减弱的艾美耳球虫系^[65]。然而,目前仅柔嫩艾美耳球虫被证实能够通过该方法成功获得减毒的鸡胚适应株^[64]。上述2种传统方法虽然能有效降低艾美耳球虫的致病性,并保留其免疫原性,但减毒株的产量显著下降,这无疑提高了球虫病疫苗的生产成本,同时也增加了基于减毒株开发重组艾美耳球虫疫苗载体的经济负担。另一方面,随着艾美耳球虫基因编辑系统的不断优化,构建艾美耳球虫一个或多个毒力基因缺失虫株,有望大幅提升其作为疫苗载体的安全性。因此,综合多组学、遗传学和分子生物学技术,挖掘和解析艾美耳球虫的关键毒力基因是未来研究的重点方向之一。

3.2 遗传操作技术提升疫苗载体的免疫原性

通过基因改造来增强低或中等免疫原性艾美耳球虫虫种(如毒害艾美耳球虫和柔嫩艾美耳球虫)的免疫原性,并降低其致病性,是开发下一代球虫病疫苗的一个极具前景的方向。经过改造的重组艾美耳球虫株系也因其更高安全性而成为理想的疫苗载体。

研究发现,疫苗载体表达的分子佐剂(如细胞因子和免疫配体)或与其共同免疫的重组蛋白/DNA亚单位疫苗,相比于未使用佐剂的疫苗,可显著增强保护性免疫^[66-67]。多项研究表明,重组艾美耳球虫表达的分子佐剂能显著增强其免疫原性。不同类型的分子佐剂有时会影响诱导的免疫应答类型。例如,表达的鸡白细胞介素-2(IL-2)在重组艾美耳球虫整个生命周期中持续分泌,可增强艾美耳球虫可溶性抗原的细胞介导免疫,但对体液免疫无显著影响或仅有轻微增强作用^[41]。此外,将免疫球蛋白 Fc 片段与 GPI (Glycosylphosphatidylinositol) 锚定结构融合至重组艾美耳球虫表面展示,也可提高其对野生型艾美耳球虫感染的保护作用^[68]。研究还发现,来源于高免疫原性艾美耳球虫的抗原可作为佐剂,提高其他艾美耳球虫的免疫原性。例如,表达巨型艾美耳球虫 profilin 蛋白的重组柔嫩艾美耳球虫可增强自身的免疫原性,从而诱导更强的细胞免疫反应,并对柔嫩艾美耳球虫感染提供更好的保护^[69]。上述研究成果表明,对艾美耳球虫进行基因改造以其作为新型疫苗载体以及安全高效的球虫病疫苗是完全可行的。

4 展 望

过去近30年中,顶复门寄生虫——艾美耳球虫的遗传操作技术及其在疫苗载体和基础生物学研究中的应用取得了飞跃式发展。目前,鸡、兔及啮齿类动物的艾美耳球虫均已成功建立瞬时转染和稳定筛选系统。实验室研究阶段已取得诸多令人鼓舞的成果,并验证了其可行性和应用前景,我国科研团队在此领域做出了引领性的贡献:据 Scopus 数据库不完全统计,我国科研团队关于艾美耳球虫疫苗载体的发文量在原生动物(包括疟原虫、弓形虫、利什曼原虫、锥虫等)作为疫苗载体的占比接近1/5。尽管如此,要实现艾美耳球虫作为高效疫苗载体或商业化疫苗的应用仍面临诸多挑战,包括但不限于:1)艾美耳球虫体外培养系统效率依然较低,这增加了疫苗构建和筛选的难度与成本^[70];2)艾美耳球虫的转染效率远低于其他顶复门寄生虫(如弓形虫或疟原虫),更不及细菌或哺乳动物细胞系,加之难以进行体外培养,依赖本动物的筛选系统增加了研发的成本和周期;3)宿主针对艾美耳球虫感染与再感染的免疫机制复杂,尚未完全阐明,基于免疫学理论的对不同类型异源病原抗原的递

呈或展示在实际设计中较难实现;4)诱导艾美耳球虫强保护性免疫反应的抗原认知不足,对外源基因的靶向定位或空间展示的指导作用较弱,需进行多次优化与提升。随着基于CRISPR/Cas9系统的基因编辑技术的成功应用,在艾美耳球虫抗原挖掘、免疫应答机制解析、耐药位点解析及其在遗传操作中的应用等方面均起到巨大的推进作用,有望突破上述技术瓶颈实现重组艾美耳球虫疫苗载体性能的提升^[28]。

另一方面,鸡的4种球虫(柔嫩艾美耳球虫、缓艾美耳球虫、毒害艾美耳球虫和堆型艾美耳球虫)均已成功建立遗传操作平台,而现阶段的研究主要集中于柔嫩艾美耳球虫和堆型艾美耳球虫为载体表达异源抗原。表达目标抗原的重组艾美耳球虫可对异源病原体提供一定程度的保护。由于不同种的艾美耳球虫寄生于肠道的不同部位,结合不同种的重组艾美耳球虫采用同时免疫、并在多个靶位点表达异源抗原的策略,有望提高疫苗的保护效果^[62]。特别是针对巨型艾美耳球虫(鸡球虫中免疫原性最强的种)建立转染平台显得尤为紧迫。

重组艾美耳球虫作为疫苗载体具有多方面优势,是开发新型兽用生物制品的可行且前景广阔的策略。目前已有部分疫苗载体(如疱疹病毒、腺病毒和沙门氏菌属)实现商业化应用,未来可参考这些成功经验,更好地优化艾美耳球虫载体与所表达抗原之间的匹配关系,包括目标抗原的选择、异源抗原的表达水平、诱导免疫反应的类型与强度,以及对异源病原体的保护效果等。尽管如此,生物技术的快速发展和持续革新将有助于克服这些障碍,推动重组艾美耳球虫作为疫苗载体的潜力实现,并为开发更多动物和人类疫苗载体开辟新路径。

参考文献 References

- [1] Beitelshes M, Li Y, Pfeifer B A. Enhancing vaccine effectiveness with delivery technology[J]. *Current Opinion in Biotechnology*, 2016, 42: 24-29
- [2] Morris S J, Sebastian S, Spencer A J, Gilbert S C. Simian adenoviruses as vaccine vectors[J]. *Future Virology*, 2016, 11(9): 649-659
- [3] Baron M D, Iqbal M, Nair V. Recent advances in viral vectors in veterinary vaccinology[J]. *Current Opinion in Virology*, 2018, 29: 1-7
- [4] Galen J E, Pasetti M F, Tennant S, Ruiz-Olvera P, Szein M B, Levine M M. *Salmonella enterica* serovar Typhi live vector vaccines finally come of age[J]. *Immunology and Cell Biology*, 2009, 87(5): 400-412
- [5] Blake D P. *Eimeria* of chickens: the changing face of an old foe[J]. *Avian Pathology*, 2025, 54(3): 267-278
- [6] Liu Q, Liu X Y, Zhao X M, Zhu X Q, Suo X. Live attenuated anticoccidial vaccines for chickens[J]. *Trends in Parasitology*, 2023, 39(12): 1087-1099
- [7] 秦梅, 汤新明, 龚玉姣, 史芳芸, 王思, 索勋. 鸡球虫病疫苗研发新思考[J]. *中国农业大学学报*, 2023, 28(6): 1-11
Qin M, Tang X M, Gong Y J, Shi F Y, Wang S, Suo X. Rethinking novel anticoccidial vaccine design[J]. *Journal of China Agricultural University*, 2023, 28(6): 1-11 (in Chinese)
- [8] Entzeroth R, Mattig F R, Werner-Meier R. Structure and function of the parasitophorous vacuole in *Eimeria* species[J]. *International Journal for Parasitology*, 1998, 28(7): 1015-1018
- [9] Vrba V, Pakandl M. Host specificity of turkey and chicken *Eimeria*: Controlled cross-transmission studies and a phylogenetic view[J]. *Veterinary Parasitology*, 2015, 208(3/4): 118-124
- [10] Shirley M W, Smith A L, Tomley F M. The biology of avian *Eimeria* with an emphasis on their control by vaccination[J]. *Advances in Parasitology*, 2005, 60: 285-330
- [11] Reid A J, Blake D P, Ansari H R, Billington K, Browne H P, Bryant J, Dunn M, Hung S S, Kawahara F, Miranda-Saavedra D, Malas T B, Mourier T, Naghra H, Nair M, Otto T D, Rawlings N D, Rivallier P, Sanchez-Flores A, Sanders M, Subramaniam C, Tay Y L, Woo Y, Wu X K, Barrell B, Dear P H, Doerig C, Gruber A, Ivens A C, Parkinson J, Rajandream M A, Shirley M W, Wan K L, Berriman M, Tomley F M, Pain A. Genomic analysis of the causative agents of coccidiosis in domestic chickens[J]. *Genome Research*, 2014, 24(10): 1676-1685
- [12] Shirley M W, Ivens A, Gruber A, Madeira A M B N, Wan K L, Dear P H, Tomley F M. The *Eimeria* genome projects: A sequence of events[J]. *Trends in Parasitology*, 2004, 20(5): 199-201
- [13] Pallesen J, Wang N S, Corbett K S, Wrapp D, Kirchdoerfer R N, Turner H L, Cottrell C A, Becker M M, Wang L S, Shi W, Kong W P, Andre E L, Kettenbach A N, Denison M R, Chappell J D, Graham B S, Ward A B, McLellan J S. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2017, 114(35): 7348-7357
- [14] Zhao D, Suo J, Liang L, Liang R, Zhou R, Ding J, Liu X, Suo X, Zhang S, Tang X. Innovative prevention and control of coccidiosis: Targeting sporogony for new control agent development[J]. *Poultry Science*, 2024, 103(12): 104246
- [15] Gao Y, Sun P, Hu D D, Tang X M, Zhang S X, Shi F Y, Yan X L, Yan W C, Shi T Y, Wang S, Zou J, Yin G W, Liu X Y, Dong H, Suo X. Advancements in understanding chicken coccidiosis: From *Eimeria* biology to innovative control strategies[J]. *One Health Advances*, 2024, 2(1): 6
- [16] Chapman H D, Cherry T E, Danforth H D, Richards G, Shirley M W, Williams R B. Sustainable coccidiosis control in poultry production: The role of live vaccines[J]. *International Journal for Parasitology*, 2002, 32(5): 617-629
- [17] Goonewardene R, Daily J, Kaslow D, Sullivan T J, Duffy P, Carter R, Mendis K, Wirth D. Transfection of the malaria parasite and expression of firefly luciferase[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1993, 90(11): 5234-5236
- [18] Kim K, Soldati D, Boothroyd J C. Gene replacement in *Toxoplasma gondii* with chloramphenicol acetyltransferase as selectable marker[J]. *Science*, 1993, 262(5135): 911-914
- [19] Kelleher M, Tomley F. Transient expression of β -galactosidase in differentiating sporozoites of *Eimeria tenella*[J]. *Molecular and Biochemical Parasitology*, 1998, 97(1/2): 21-31
- [20] Hao L L, Liu X Y, Zhou X Y, Li J D, Suo X. Transient transfection of

- Eimeria tenella* using yellow or red fluorescent protein as a marker[J]. *Molecular and Biochemical Parasitology*, 2007, 153(2): 213-215
- [21] Duan C H, Hu D D, Tang X M, Suo J X, Wang S, Zhang S X, Tao G R, Li C, Wang C Y, Gu X L, Tang X L, Huang G P, Xiang B Q, Wu S Q, Ben Mamoun C, Suo X, Liu X Y. Stable transfection of *Eimeria necatrix* through nucleofection of second generation merozoites [J]. *Molecular and Biochemical Parasitology*, 2019, 228: 1-5
- [22] Liu X Y, Shi T Y, Ren H B, Su H L, Yan W C, Suo X. Restriction enzyme-mediated transfection improved transfection efficiency *in vitro* in Apicomplexan parasite *Eimeria tenella* [J]. *Molecular and Biochemical Parasitology*, 2008, 161(1): 72-75
- [23] Clark J D, Billington K, Bumstead J M, Oakes R D, Soon P E, Sopp P, Tomley F M, Blake D P. A toolbox facilitating stable transfection of *Eimeria* species[J]. *Molecular and Biochemical Parasitology*, 2008, 162(1): 77-86
- [24] Liu J, Shi F Y, Zhang Y Y, Tang X M, Wang C Y, Gao Y, Suo J X, Yu Y L, Chen L L, Zhang N, Sun P, Liu X Y, Suo X. Evidence of high-efficiency cross fertilization in *Eimeria aceroulina* revealed using two lines of transgenic parasites[J]. *International Journal for Parasitology*, 2023, 53(2): 81-89
- [25] Shi T Y, Tao G R, Bao G L, Suo J X, Hao L L, Fu Y, Suo X. Stable transfection of *Eimeria intestinalis* and investigation of its life cycle, reproduction and immunogenicity[J]. *Frontiers in Microbiology*, 2016, 7: 807
- [26] Kurth M, Entzeroth R. Reporter gene expression in cell culture stages and oocysts of *Eimeria nieschulzi* (Coccidia, Apicomplexa) [J]. *Parasitology Research*, 2009, 104(2): 303-310
- [27] Pastor-Fernández I, Pegg E, Macdonald S E, Tomley F M, Blake D P, Marugán-Hernández V. Laboratory growth and genetic manipulation of *Eimeria tenella*[J]. *Current Protocols in Microbiology*, 2019, 53(1): e81
- [28] Li Y R, Suo J X, Liang R Y, Liang L, Liu X Y, Ding J B, Suo X, Tang X M. Genetic manipulation for the non-model protozoan *Eimeria*: Advancements, challenges, and future perspective[J]. *iScience*, 2025, 28(3): 112060
- [29] Qin M, Liu X Y, Tang X M, Suo J X, Tao G R, Suo X. Transfection of *Eimeria mitis* with yellow fluorescent protein as reporter and the endogenous development of the transgenic parasite[J]. *PLoS One*, 2014, 9(12): e114188
- [30] Zhang S X, Tang X M, Wang S, Shi F Y, Duan C H, Bi F F, Suo J X, Hu D D, Liu J, Wang C Y, Suo X, Liu X Y. Establishment of recombinant *Eimeria aceroulina* expressing multi-copies M2e derived from avian influenza virus H9N2[J]. *Vaccines*, 2021, 9(7): 791
- [31] Tao G R, Shi T Y, Tang X M, Duszynski D W, Wang Y Z, Li C, Suo J X, Tian X L, Liu X Y, Suo X. Transgenic *Eimeria magna* Pêrard, 1925 displays similar parasitological properties to the wild-type strain and induces an exogenous protein-specific immune response in rabbits (*Oryctolagus cuniculus* L)[J]. *Frontiers in Immunology*, 2017, 8: 2
- [32] Tang X M, Liu X Y, Tao G R, Qin M, Yin G W, Suo J X, Suo X. "Self-cleaving" 2A peptide from porcine teschovirus-1 mediates cleavage of dual fluorescent proteins in transgenic *Eimeria tenella*[J]. *Veterinary Research*, 2016, 47(1): 68
- [33] Jongeo A M, Ting L M, Thathy V, Mota M M, Kim K. Improved transfection and new selectable markers for the rodent malaria parasite *Plasmodium yoelii*[J]. *Molecular and Biochemical Parasitology*, 2006, 146(2): 242-250
- [34] Di Cristina M, Ghouze F, Kocken C H, Naitza S, Cellini P, Soldati D, Thomas A W, Crisanti A. Transformed *Toxoplasma gondii* tachyzoites expressing the circumsporozoite protein of *Plasmodium knowlesi* elicit a specific immune response in rhesus monkeys[J]. *Infection and Immunity*, 1999, 67(4): 1677-1682
- [35] Sun P, Zhang Y Y, Wang C Y, Hu D D, Liu J, Chen L L, Shi F Y, Tang X M, Hao Z K, Suo J X, Yu Y L, Suo X, Liu X Y. *EtcPRS*^{Mut} as a molecular marker of halofuginone resistance in *Eimeria tenella* and *Toxoplasma gondii* [J]. *iScience*, 2023, 26(4): 106334
- [36] 毕菲菲, 郝振凯, 孙露, 于咏兰, 刘贤勇, 索勋. 基于全基因组重测序及等位基因频率解析柔嫩艾美耳球虫对地克珠利耐药分子机制的初步研究[J]. 中国兽医杂志, 2022, 58(8): 1-6
- Bi F F, Hao Z K, Sun P, Yu Y L, Liu X Y, Suo X. Preliminary study of molecular mechanisms of diclazuril resistance in *Eimeria tenella* based on whole-genome resequencing and allele frequency analysis [J]. *Chinese Journal of Veterinary Medicine*, 2022, 58(8): 1-6 (in Chinese)
- [37] Sun P, Wang C Y, Xie F J, Chen L L, Zhang Y Y, Tang X M, Hu D D, Gao Y, Zhang N, Hao Z K, Yu Y L, Suo J X, Suo X, Liu X Y. The F204S mutation in adrenodoxin oxidoreductase drives salinomycin resistance in *Eimeria tenella*[J]. *Veterinary Research*, 2024, 55(1): 170
- [38] Sun P, Wang C Y, Zhang Y Y, Tang X M, Hu D D, Xie F J, Hao Z K, Suo J X, Yu Y L, Suo X, Liu X Y. Transcriptome profile of halofuginone resistant and sensitive strains of *Eimeria tenella* [J]. *Frontiers in Microbiology*, 2023, 14: 1141952
- [39] Gu X L, Fang S F, Liu H B, Shi Y B, Zhang Y Y, Wang P, Cui P, Tang X M. Genomic insights into monensin resistance development in *Eimeria tenella*[J]. *Frontiers in Veterinary Science*, 2025, 12: 1459791
- [40] 郝振凯, 汤新明, 张媛媛, 谢福杰, 陈君敏, 索静霞, 索勋, 刘贤勇. 利用二代测序技术对柔嫩艾美耳球虫 T-DNA 整合位点的分析及鉴定[J]. 中国农业大学学报, 2023, 28(8): 183-190
- Hao Z K, Tang X M, Zhang Y Y, Xie F J, Chen J M, Suo J X, Suo X, Liu X Y. Analysis and identification of T-DNA insertion sites in transgenic *Eimeria tenella*[J]. *Journal of China Agricultural University*, 2023, 28(8): 183-190 (in Chinese)
- [41] Li Z R, Tang X M, Suo J X, Qin M, Yin G W, Liu X Y, Suo X. Transgenic *Eimeria mitis* expressing chicken interleukin 2 stimulated higher cellular immune response in chickens compared with the wild-type parasites [J]. *Frontiers in Microbiology*, 2015, 6: 533
- [42] Liu X Y, Zou J, Yin G W, Su H L, Huang X X, Li J N, Xie L, Cao Y Q, Cui Y J, Suo X. Development of transgenic lines of *Eimeria tenella* expressing M2e-enhanced yellow fluorescent protein (M2e-EYFP) [J]. *Veterinary Parasitology*, 2013, 193(1/3): 1-7
- [43] Yan W C, Liu X Y, Shi T Y, Hao L L, Tomley F M, Suo X. Stable transfection of *Eimeria tenella*: Constitutive expression of the YFP-YFP molecule throughout the life cycle [J]. *International Journal for Parasitology*, 2009, 39(1): 109-117
- [44] Tang X M, Suo J X, Liang L, Duan C H, Hu D D, Gu X L, Yu Y L, Liu X Y, Cui S J, Suo X. Genetic modification of the protozoan *Eimeria tenella* using the CRISPR/Cas9 system[J]. *Veterinary Research*, 2020, 51(1): 41
- [45] Hu D D, Tang X M, Ben Mamoun C, Wang C Y, Wang S, Gu X L, Duan C H, Zhang S X, Suo J X, Deng M E, Yu Y L, Suo X, Liu X Y. Efficient single-gene and gene family editing in the apicomplexan parasite *Eimeria tenella* using CRISPR-Cas9 [J]. *Frontiers in Bioengineering and Biotechnology*, 2020, 8: 128
- [46] Cheng P P, Zhang Z H, Yang F Y, Cai S, Wang L N, Wang C M, Wang M, Liu Y C, Fei C Z, Zhang L F, Xue F Q, Gu F. FnCas12a/crRNA-mediated genome editing in *Eimeria tenella*[J]. *Frontiers in Genetics*, 2021, 12: 738746
- [47] Zou J, Liu X Y, Shi T Y, Huang X X, Wang H Y, Hao L L, Yin G W, Suo X. Transfection of *Eimeria* and *Toxoplasma* using heterologous regulatory sequences[J]. *International Journal for Parasitology*, 2009, 39(11): 1189-1193
- [48] Yin G W, Liu X Y, Zou J, Huang X X, Suo X. Co-expression of reporter

- genes in the widespread pathogen *Eimeria tenella* using a double-cassette expression vector strategy[J]. *International Journal for Parasitology*, 2011, 41(8): 813-816
- [49] Yin G W, Qin M, Liu X Y, Suo J X, Suo X. Expression of *Toxoplasma gondii* dense granule protein7 (GRA7) in *Eimeria tenella*[J]. *Parasitology Research*, 2013, 112(5): 2105-2109
- [50] Tang X M, Liu X Y, Yin G W, Suo J X, Tao G R, Zhang S X, Suo X. A novel vaccine delivery model of the apicomplexan *Eimeria tenella* expressing *Eimeria maxima* antigen protects chickens against infection of the two parasites[J]. *Frontiers in Immunology*, 2018, 8: 1982
- [51] Tang X M, Yin G W, Qin M, Tao G R, Suo J X, Liu X Y, Suo X. Transgenic *Eimeria tenella* as a vaccine vehicle: Expressing TgSAG1 elicits protective immunity against *Toxoplasma gondii* infections in chickens and mice[J]. *Scientific Reports*, 2016, 6: 29379
- [52] Shi T Y, Yan W C, Ren H B, Liu X Y, Suo X. Dynamic development of parasitophorous vacuole of *Eimeria tenella* transfected with the yellow fluorescent protein gene fused to different signal sequences from apicomplexan parasites[J]. *Parasitology Research*, 2009, 104(2): 315-320
- [53] Yu Y, Zhang S X, Duan C H, Crouch C, Suo J X, Tang X M, Liu X Y, Liu J, Bruton B, Tarpey I, Suo X. Developing efficient strategies for localizing the enhanced yellow fluorescent protein subcellularly in transgenic *Eimeria* parasites[J]. *Scientific Reports*, 2024, 14: 4851
- [54] Guo Q B, Yu Y, Suo J X, Tang X M, Zhang S X, Crouch C, Bruton B, Tarpey I, Liu X Y, Zhao G H, Suo X. Oral delivery of *Eimeria aceroulina* transfected sequentially with two copies of the VP2 gene induces immunity against infectious bursal disease virus in chickens[J]. *Frontiers in Veterinary Science*, 2024, 11: 1367912
- [55] Huang X X, Zou J, Xu H Q, Ding Y, Yin G W, Liu X Y, Suo X. Transgenic *Eimeria tenella* expressing enhanced yellow fluorescent protein targeted to different cellular compartments stimulated dichotomic immune responses in chickens[J]. *Journal of Immunology*, 2011, 187(7): 3595-3602
- [56] Jonscher E, Erdbeer A, Günther M, Kurth M. Two COWP-like cysteine rich proteins from *Eimeria nieschulzi* (coccidia, apicomplexa) are expressed during sporulation and involved in the sporocyst wall formation[J]. *Parasites & Vectors*, 2015, 8: 395
- [57] Tang X M, Liu X Y, Suo X. Towards Innovative design and application of recombinant *Eimeria* as a vaccine vector[J]. *Infection and Immunity*, 2020, 88(5): e00861-19
- [58] Marugan-Hernandez V, Cockle C, MacDonald S, Pegg E, Crouch C, Blake D P, Tomley F M. Viral proteins expressed in the protozoan parasite *Eimeria tenella* are detected by the chicken immune system[J]. *Parasites & Vectors*, 2016, 9(1): 463
- [59] Yu Y, Tang X M, Duan C H, Suo J X, Crouch C, Zhang S X, Liu X Y, Liu J, Bruton B, Tarpey I, Suo X. Microneme-located VP2 in *Eimeria aceroulina* elicits effective protective immunity against infectious bursal disease virus[J]. *Infection and Immunity*, 2024, 92(2): e0045623
- [60] Pastor- Fernández I, Kim S, Billington K, Bumstead J, Marugán-Hernández V, Küster T, Ferguson D J P, Vervelde L, Blake D P, Tomley F M. Development of cross-protective *Eimeria*-vectored vaccines based on apical membrane antigens[J]. *International Journal for Parasitology*, 2018, 48(7): 505-518
- [61] Clark J D, Oakes R D, Redhead K, Crouch C F, Francis M J, Tomley F M, Blake D P. *Eimeria* species parasites as novel vaccine delivery vectors: Anti-*Campylobacter jejuni* protective immunity induced by *Eimeria tenella*-delivered CjaA[J]. *Vaccine*, 2012, 30(16): 2683-2688
- [62] Tang X M, Wang C Y, Liang L, Hu D D, Zhang S X, Duan C H, Suo J X, Liu X Y, Suo X, Cui S J. Co-immunization with two recombinant *Eimeria tenella* lines expressing immunoprotective antigens of *E. maxima* elicits enhanced protection against *E. maxima* infection[J]. *Parasites & Vectors*, 2019, 12(1): 347
- [63] Jeffers T K. Attenuation of *Eimeria tenella* through selection for precociousness[J]. *The Journal of Parasitology*, 1975, 61(6): 1083-1090
- [64] Shirley M W. Development of a live attenuated vaccine against coccidiosis of poultry[J]. *Parasite Immunology*, 1989, 11(2): 117-124
- [65] Long P L. Development of *Eimeria tenella* in avian embryos[J]. *Nature*, 1965, 208(5009): 509-510
- [66] Suschak J J, Williams J A, Schmaljohn C S. Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity[J]. *Human Vaccines & Immunotherapeutics*, 2017, 13(12): 2837-2848
- [67] Rhee E G, Kelley R P, Agarwal I, Lynch D M, Porte A L, Simmons N L, Clark S L, Barouch D H. TLR4 ligands augment antigen-specific CD8⁺ T lymphocyte responses elicited by a viral vaccine vector[J]. *Journal of Virology*, 2010, 84(19): 10413-10419
- [68] Qin M, Tang X M, Yin G W, Liu X Y, Suo J X, Tao G R, El-Ashram S, Li Y, Suo X. Chicken IgY Fc expressed by *Eimeria mitis* enhances the immunogenicity of *E. mitis*[J]. *Parasites & Vectors*, 2016, 9: 164
- [69] Tang X M, Suo J X, Li C, Du M Z, Wang C Y, Hu D D, Duan C H, Lyu Y L, Liu X Y, Suo X. Transgenic *Eimeria tenella* expressing profilin of *Eimeria maxima* elicits enhanced protective immunity and alters gut microbiome of chickens[J]. *Infection and Immunity*, 2018, 86(9): e00888-17
- [70] 谢月华, 谢福杰, 索勋, 闫文朝. 3D细胞培养系统在顶复门原虫研究中的应用[J]. 畜牧兽医学报, 2025, 56(4): 1540-1548
- Xie Y H, Xie F J, Suo X, Yan W C. Application of the 3D cell culture system in the study of apicomplexa protozoa[J]. *Acta Veterinaria et Zootechnica Sinica*, 2025, 56(4): 1540-1548 (in Chinese)

责任编辑: 秦梅



第一作者简介: 汤新明, 博士, 硕士研究生导师, 中国农业科学院北京畜牧兽医研究所副研究员, 动物生物安全与公共卫生防控科技创新团队科研骨干, 宠物疫病防控研究方向负责人。研究方向为重要动物寄生虫病防控、新型载体疫苗设计和病原的发育调控机理。兼任《中国农业科学》青年编委、《中国农业大学学报》栏目主编、*Animal Research and One Health* 编委。获中国农业科学院青年创新专项资助, 主持国家自然科学基金、国家重点研发计划子课题、北京市自然科学基金、中国博士后基金等国家和省部级科研项目10项。参编/译中英文著作3部, 授权发明专利10余件, 发表SCI论文40余篇。