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A review of Extracellular Vesicles in Parasitic Diseases: Mechanisms and Translational Opportunities

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Abstract

Extracellular vesicles (EVs) are membrane-delimited particles secreted by cells in all domains of life, and are now widely regarded as core mediators of intercellular communication in health and disease. Within the field of parasitology, EV studies have deepened our understanding of how protozoa and helminths communicate with host tissue, evade immune responses, and orchestrate parasite-to-parasite signalling. Concurrently, this review reflects on recent results on the biogenesis and molecular cargo of EVs and reiterates methodological rigour and standardization as recommended by the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines. We explore mechanisms through EVs derived from major protozoan pathogens (Plasmodium spp., Trypanosoma brucei, Leishmania spp., and Giardia duodenalis) and from helminths in showing how vesicular proteins, lipids, and small RNAs drive host innate and adaptive responses. We conclude by reviewing host EVs produced by parasitic infection in terms of inflammatory signaling, endothelial activation, anemia, and tissue remodeling. The translational components examine EVs as candidate biomarkers, vaccine components, and delivery platforms and note technical and biological limitations in this regard, especially EV heterogeneity, co-isolation of contaminants, and variability between in vitro and in vivo systems. Last, we provide a regional perspective with reference to recent reports of parasitological surveys and molecular diagnostics conducted in Kirkuk Province (Iraq), presenting ongoing burdens of intestinal parasitism and zoonotic transmission at the human-animal interface. Integrating these parasite-EV examinations with robust reporting mechanisms and appropriate sampling methods guided by the science of medicine can facilitate a successful translation of this mechanistic insight to a comprehensive idea of diagnostics and intervention.

Keywords: Extracellular Vesicles; Exosomes; Microvesicles; Host-Parasite Interaction; Immunomodulation; Protozoa; Helminths; Biomarkers; Kirkuk; Iraq

1. Introduction

Parasitic disease is one of the leading causes of global morbidity and the burdens are often concentrated in resource constrained settings in which environmental exposure, poor sanitation facilities, and limited access to diagnostic tests intersect. Historical epidemiological work from Northern Iraq helps to set the context: sampling in Kirkuk Province has shown the presence of high concentration levels of gastrointestinal parasites circulating in stray dogs and cats (reservoirs capable of contaminating soil and water) and similar high concentration (as well) of gastrointestinal parasites in ruminants, reflecting the importance of zoonotic and food-chain routes (Hassan & Barzinji, 2018). In these conditions, intestinal protozoa such as Entamoeba histolytica, Cryptosporidium parvum and Giardia lamblia were identified in immunologically sensitive communities (e.g. children with type 1 diabetes) from microscopy and antigen and PCR based methods (Raza *et al*., 2025). Meanwhile, molecular screening for Toxoplasma gondii in women diagnosed with breast cancer in Kirkuk City has been reported, pointing to the importance of sensitive molecular tools for parasite

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detection in special clinical settings (Mahmood & Rezaig, 2019). Under this epidemiological setting, extracellular vesicles have emerged as the mechanistic intermediary between parasites and host biology. EVs are lipid bilayer-delimited heterogeneous components with proteins, lipids, and nucleic acids that can be ingested by recipient cells for cell phenotypic modification (van Niel *et al*., 2018; Mathieu *et al*., 2019). Investigations in various parasitic systems reveal that EV production by parasites and production of EVs from infected host cells that possess altered cargo profiles has the potential to influence immune regulation, vascular pathology, or tissue microenvironments (Marcilla *et al*., 2014; Montaner *et al*., 2014). This review aims to present a summary of EV biology with specific focus on parasitic infections but also technical standards and reproducibility; and translational challenges relevant for the diagnosis and therapy of EVs.

1.1. Burden of parasitic diseases and regional context

Parasitic transmission is influenced by ecology, socioeconomics, and animal–human interfaces. In Kirkuk Province, a cross-sectional survey of stray dogs and cats found extremely high overall infection rates of gastrointestinal parasites (helminths and protozoa), which could indicate sustained environmental contamination and potential for zoonotic spillover (Hassan & Barzinji, 2018). Livestock is also a focal point of concern: ruminants are important reservoirs of gastrointestinal helminths and protozoa with potential direct contribution to reduced productivity, but also as sources of infection in other species and humans through shared habitat use and husbandry (Hassan & Barzinji, 2018). These studies provide evidence of the necessity for integrated management of parasitic diseases by combining their veterinary, environmental, and clinical approaches. Clinical observation provides additional insight. A 2025 study carried out in Kirkuk revealed the prevalence of intestinal parasite infection in children with type 1 diabetes based on stool analysis and highlighted the importance of integrating microscopy with immunochromatographic and PCR assays for assessing detection methods, specifically with Cryptosporidium (Raza *et al*., 2025). In a Kirkuk study, real-time PCR was used to detect Toxoplasma gondii DNA in sera of women with breast cancer; molecular diagnostics is thus relevant to specific clinical procedures (Mahmood & Rezaig, 2019). These findings from the region drive the need for novel field-specific biomarkers. The EVs themselves are promising here. They hold hope for recovery from biofluids of interest and can aggregate both pathogen- and host-derived signatures (Menezes & Tasca, 2024).

2. Overview of extracellular vesicle biology and terminology

EVs can be further divided into those known as exosomes (derived from the endosomal system via multivesicular bodies) and those called microvesicles (shed directly from the plasma membrane); however size-based naming alone cannot identify the EV subtypes (van Niel *et al*., 2018). EV cargo is not a random cytosol snapshot, but selective sorting mechanisms enrich particular proteins, lipids, and RNAs, and recipient-cell uptake can take place through endocytosis, phagocytosis, macropinocytosis, or direct membrane fusion (Mathieu *et al*., 2019). Because EV populations are heterogeneous and often co-isolate with protein aggregates, lipoproteins, and other extracellular particles, we require careful methodological design and dissemination. MISEV guidelines also give communities a consensus on minimum experimental and reporting parameters. In MISEV2018, it suggested a harmonisation of EV definition, characterization, and functional claims, EV-associated markers to be displayed, contaminants to be tested, and orthogonal methods to be utilized for characterization (Théry *et al*., 2018). MISEV2023 further extended guidance toward numerous contexts and technical levels with a greater focus on rigor, transparency, and fit-for-purpose processes as the field enters clinical translation (Welsh *et al*., 2024).

3. Methodological approaches and quality control in parasite-EV studies

In parasitology, isolation typically entails the use of differential centrifugation with ultracentrifugation, density-gradient separation, and size-exclusion chromatography. For parasite cell culture systems, media composition, serum EV depletion, and parasite life-cycle stage can significantly impact recoverable EV populations, emphasizing the importance of clear information about culture conditions and sampling timepoints (Théry *et al*., 2018; Welsh *et al*., 2024). Characterization usually includes particle sizing (e.g., nanoparticle tracking analysis), imaging (transmission electron microscopy), and detection of EV-associated markers. However, parasite EV marker panels are generally less well standardized than mammalian ones, and recent literature highlights the requirement for organism-specific EV marker validation, particularly kinetoplastids and apicomplexans (Marcilla et al., 2014; Gabriel *et al*., 2021). Functional studies require controls which distinguish EV-induced effects from soluble secreted factors and co-isolated contaminants. MISEV suggests that dose-response assessment, uptake by recipient cells, and EV depletion/disruption controls are included when possible in the assessments (Théry *et al*., 2018; Welsh *et al*., 2024). For parasite-host systems, other confounders could involve microbial co-infections in clinical samples, hemolysis within blood-derived EVs, and the high levels of host lipoproteins. Such standardized workflows and clinically relevant region-specific sampling, including

stool- and plasma-based diagnostics, for use in Kirkuk-based studies, should enhance the interpretability and translational relevance of EV studies (Raza *et al*., 2025).

4. Protozoan parasite-derived EVs: mechanisms and host modulation

Protozoan parasites possess EVs throughout many taxa and can contain surface molecules, enzymes, lipids, and small RNAs that facilitate host immune and barrier function modification. For protozoan infections, EVs are increasingly viewed as vehicles for immune modulation—either dampening protective responses or amplifying inflammation driving the distribution or survival of the parasite itself (Marcilla *et al*., 2014; Montaner *et al*., 2014; Menezes & Tasca, 2024). Below, we summarize evidence from prominent model systems.

4.1. Plasmodium spp. and malaria-associated EVs

EVs produced from infected erythrocytes and host immune cells are involved in both parasite communication and host immunopathology in malaria. Review-level syntheses indicate active involvement of EVs in inflammatory cascades and endothelial activation, mediated by cytokine signaling and coagulation-related pathways (Babatunde *et al*., 2020), and involved in disease severity. Studies in malaria have shown that EVs may act as an intermediary in parasite-to-parasite signaling, and EV cargo has been proposed as a potential biomarker for disease staging and prognosis (Babatunde *et al*., 2020; Menezes & Tasca, 2024). Therefore, to do diagnostic translation it requires consideration of pre-analytical parameters, e.g., hemolysis and co-isolation of lipoproteins, as well as the results of broader guidance on EV methodology (Théry *et al*., 2018; Welsh *et al*., 2024).

4.2. African trypanosomes and EV-mediated virulence factor transfer

Trypanosoma brucei is perhaps the most interesting exemplar of the pathophysiological relevance of parasite EVs. Based on previous work in the literature, bloodstream trypanosomes create membranous nanotubes that produce EVs containing flagellar proteins and virulence-associated components. These EVs may combine with other trypanosomes and mammalian erythrocytes to undertake erythrocyte remodeling and rapid clearance, both of which are associated with anemia (Szempruch *et al*., 2016). They were consistent with a model where EVs function as organelles that carry non-genetically inherited virulence factors between parasites and also as the effector mechanisms driving pertinent clinical host pathology. These findings also indicate the validation of the EV uptake and influence in experimental conditions using the appropriate controls and orthogonal analysis (Théry *et al*., 2018; Welsh *et al*., 2024).

4.3. Leishmania spp.: exosomal research frameworks and immunomodulation

Leishmania spp. secrete extracellular vesicles (otherwise known as Leishmania EVs or LEVs), which can influence macrophage signaling and parasite survival within phagolysosomal environments. An extensive methodology review of these approaches yielded practical recommendations for LEV analysis, emphasizing the requirement for harmonized isolation, characterization, and reporting relevant to Leishmania biology and experimental systems (Gabriel *et al*., 2021). Under this framework, LEVs are introduced as carriers of parasite molecules capable of shaping innate immune responses and potentially contributing to disease phenotypes. As with other parasite systems, LEV functional claims benefit from strong evidence of vesicle-associated activity distinct from soluble secreted products, aligned with community standards (Théry *et al*., 2018; Welsh *et al*., 2024).

4.4. Giardia duodenalis: EVs, adhesion, and inflammatory signaling

Giardia is an extracellular protozoan which plays a role in communicating with intestinal epithelial cells, resulting in malabsorption and diarrhea. EVs have been identified as yet an additional level of host-parasite communication. Recent in vitro studies have also determined Giardia secreted products for epithelial stimulation (Ma'ayeh *et al*., 2017) and reported that secreted molecules including EVs-related elements modulate host cell responses. In experimental studies as well, the EVs from Giardia have been found to be internalized by macrophages and to stimulate activation of inflammatory cytokines and activate IL-1 through TLR2 and NLRP3 inflammasome-related signalling pathways (Zhao *et al*., 2021). Also, the pharmacological blockade of the peptidylarginine deiminase activity resulted in inhibition of large extracellular vesicles of Giardia generation and impaired parasite-cell adhesion, associated with EV biogenesis with functional characteristics that are related to infection dynamics (Gavinho *et al*., 2020). Collectively, these accounts lend evidence for a Giardia EV as a host immune activating and parasite attachment mediator and a molecular target in a model.

5. Helminth-derived EVs: immune regulation and vaccine-relevant cargo

These helminths form excretory/secretory (ES) products that shape host immunity while allowing chronic persistence. EVs have now been recognized as key components of ES mixtures, packaging immune modulators in a format that protects labile molecules and promotes targeted delivery (Drurey & Maizels, 2021). Based on their synthesis of available data, Drurey and Maizels highlight that helminth EVs can carry proteins and RNAs capable of modulating multiple layers of the immune response, including innate sensing and T-cell polarization, and discuss the potential of targeting EV pathways in therapeutic strategies (Drurey & Maizels, 2021). Another open-access review emphasizes internalization of helminth EVs by host cells, which can exert variable effects depending on the parasite species and recipient cell context, with implications for biomarker discovery and vaccine development (Sánchez-López *et al*., 2021). A recent Frontiers work suggests that zoonotic helminth EV research is rapidly expanding and calls for systematic functional studies and comparative approaches to link EV composition with lifecycle biology and immunopathology (Mikhailov *et al*., 2024).

6. Host-derived EVs during parasitic infection

Parasitic infection alters host EV release and cargo, causing these changes to provoke inflammation, coagulation, vascular dysfunction, and tissue remodeling. Cell-specific cross-talk pertinent in the malaria scenario has proposed that host and parasite-modulated EVs could drive disease-associated factors like inflammatory signaling and endothelial activation (Babatunde *et al*., 2020). Moreover in a broader parasitic context, EVs have been revealed to harbor host-derived cytokine-related signals and regulatory RNAs associated with immune cell recruitment and activation (Montaner *et al*., 2014). Nonetheless, the comparison between parasite-derived EVs and host EVs in clinical specimens continues to be difficult because of the need for species-specific markers, prudent fractionation methods, and clear reporting of potential contaminants in the manner proposed by MISEV (Théry *et al*., 2018; Welsh *et al*., 2024).

7. EV cargo as diagnostic and prognostic biomarker material

EV cargo is particularly attractive for diagnostics, because EVs can concentrate nucleic acids and proteins, protect them from degradation, and circulate within accessible biofluids such as blood, urine, saliva, and stool. Parasite- and host-derived EV signatures could be considered, in recent reviews on parasitic diseases (Menezes & Tasca, 2024), as minimally invasive biomarkers if they can be coupled with sensitive molecular assays. This potential is particularly relevant in settings where mixed infections and intermittent parasite shedding reduce the sensitivity of conventional microscopy. At the clinical level in Kirkuk, the multi-method diagnostic strategy can improve the detection of intestinal parasites and protozoa in vulnerable cohorts (Raza *et al*., 2025) and the implementation of molecular detection of Toxoplasma DNA in local laboratory workflows (Mahmood & Rezaig, 2019). EV-based biomarkers would best support these approaches with stable, reproducible signals associated with parasite activity or host response. This would entail stringent pre-analytical handling, transparent reporting of isolation and characterization methods, and validation within independent cohorts (Théry *et al*., 2018; Welsh *et al*., 2024).

8. EVs as vaccine components and delivery platforms

EVs are studied as potential vaccine antigens and carrier molecules for immunomodulatory agents. In helminth paradigms, EV-associated proteins and RNAs have been proposed as candidate targets or components of vaccine strategies, and the ability of EVs to package multiple immune modulators may be advantageous for shaping protective responses (Drurey & Maizels, 2021; Sánchez-López *et al*., 2021). Mechanistic studies in trypanosomes of protozoa demonstrate that EVs also possess virulence factors that modulate host cells; as such, EV components can act as antigenic targets or indicators of pathogenic processes (Szempruch *et al*., 2016). Indeed, a methodological review on Leishmania suggests the necessity for further standardization of LEVs, and comparative profiling of the same, so that viable testing of vaccine-relevant hypotheses among strains and among models may occur (Gabriel *et al*., 2021). In these environments, translational development will also have to take into account EV heterogeneity, production scalability, safety evaluation, and adherence to standardized reporting (Théry *et al*., 2018; Welsh *et al*., 2024).

9. Key challenges and knowledge gaps

Although progress has been swift, the synthesis and translation encounter some challenges. One, EV heterogeneity impairs the ability to compare studies; sample matrices, for example, harbor elevated levels of contaminants, including lipoproteins, which hinders their isolation. Second, parasite-specific EV markers have not fully been characterized across many taxa and it's often challenging to distinguish the parasite EVs from the host EVs in clinical specimens. Third,

functional claims are based on in vitro systems that in and of themselves may not reproduce the in vivo microenvironment in every case. E.g., Giardia EV signaling is dependent on recipient cell and culture conditions, and pharmacologic perturbations on EV production must be carefully controlled for off-target effects (Gavinho *et al*., 2020; Zhao *et al*., 2021). Fourth, the strict pre-analytical manipulation and quantification and replication across cohorts are all considered key for clinical translation, and are central to MISEV frameworks (Théry et al., 2018; Welsh et al., 2024). In addition, at the regional level the integration of EV studies by integrating them with already existing surveillance projects (e.g., studying zoonotic parasites in stray animals and intestinal parasites in susceptible human populations) from a population-based approach in Kirkuk would enable evaluation of EV candidates for possible biomarkers against real-world in-the-wild infection spectra and co-infection datasets (Hassan & Barzinji, 2018; Raza *et al*., 2025).

10. Future directions

Next steps would probably include (i) standardization of parasite-EV isolation and reporting in close adherence to MISEV recommendations; (ii) building parasite-specific marker panels for EV enrichment and assured attribution of EV origin; (iii) using multi-omics to distinguish conserved EV cargo modules from those specific to particular species; and (iv) strengthening associations between mechanistic studies and clinically anchored cohorts. EV identification paired with locally focused molecular diagnostics in regional labs, such as with the recent works in Kirkuk on PCR-based workflows for Cryptosporidium and Toxoplasma, may accelerate the rapid validation of EV-associated nucleic acids as effective biomarkers (Mahmood & Rezaig, 2019; Raza *et al*., 2025). Lastly, the comparison between zoonotic cycles, including animal reservoirs and humans, could help with the understanding of evolving EV-mediated signaling in the hosts and could assess whether EV signatures might be able to predict outbreak or hot spot points of transmission (Hassan & Barzinji, 2018; Menezes & Tasca, 2024).

11. Conclusions

EVs are central to several of the modern paradigms of parasitic infection that serve to facilitate the transport of proteins and RNAs, influencing host immunity, tissue pathology, and parasite adaptation. Research from malaria, trypanosomes, Leishmania, Giardia, and helminths has indicated that EVs can serve as mediators for inflammation, immune suppression, adhesion, and virulence factor dissemination. At the same time, the diversity of EV populations and complexity of clinical matrices necessitate thoughtful methods and transparent reporting as dictated by MISEV. Leveraging standardized EV workflows when integrated with locally applicable parasitological surveillance and molecular diagnostics—as demonstrated by the Kirkuk Province studies—the field can advance to strong EV-based biomarkers and more testable vaccine and therapeutic concepts.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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