Rabbit Leishmaniasis Vaccination: Exploring Novel Antigens and Adjuvants for Veterinary Use

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Abstract

Leishmaniasis is a parasitic disease which is caused by Leishmania spp and transmitted through sandflies that are affecting humans and animals globally. Vaccination efforts focus on Novel antigens and Adjuvants to enhance protective immunity especially in veterinary specimens like Rabbits which are emerging as significant reservoirs for Leishmania infantum. Protein based antigens such as KMP-11 and heat shock proteins alongside glycan-based molecules like lipophosphoglycan in eliciting robust Th1 responses and CD+8 T-cell activation. Adjuvants including Alum enhance immunogenicity by promoting antigens presentation and cellular immunity. In Rabbits symptoms like skin lesions anemia and immunosuppression are highlighting the need for effective preventive measures and emerging vaccines strategies. Advances in antigens discovery and adjuvants formulation are crucial to control Leishmaniasis in rabbits and zoonotic transmission risks reduction.

Keywords: Leishmaniasis, Rabbit, Th1 immune response, Protein based KMP-11, Sandfly, Adjuvants (Aluminium Hydroxide)

Introduction

‘’ Leishmaniasis refers to a collection of protozoan parasites of the genus Leishmania causing diseases which are spread through the bites of phlebotomine sand flies (Usman et al., 2023). The clinical symptoms of Leishmania infections vary widely, ranging from skin and mucous membrane lesions to potentially fatal outcomes (Talukder et al., 2024)’’. Leishmaniasis is recognized as an emerging disease with Leishmania species which contribute to significant illness and death across extensive regions of old and new world (Yadav et al., 2023). It has an annual incidence of 0.9 to 1.7 million cases and occurs on every continent except Australia and Antarctica of this illness (Agura, 2024). The disease is endemic in 98 countries, placing 350 million people at risk of infection and resulting in 20,000 to 40,000 deaths annually (Gupta et al., 2023). The rise in Leishmania HIV co-infection has driven its emergence in western world (Burki, 2022). Leishmaniasis causes an estimated 2.4 million Disability-Adjusted Life Years and World Health Organization (WHO) has classified it as category I Neglected Tropical Disease (NTD) (Malik et al., 2024; Thakur, 2020).

Historical Evidence

Leishmaniasis caused by Leishmania Protozoan parasites is the second most prevalent parasitic disease. Recognized as a significant tropical disease by WHO, it has historical references with Arab physicians like Avicenna describing it as 'Balkh sore' and Indian physicians naming visceral Leishmaniasis (VL) as Kala-Azar (black fever) (McDowell & Robichaud, 2024). This disease was first observed in 1824 (Bhunia et al., 2020). Captain Donovan and Major Ross identified the causative organisms as Leishmania donovani by linking it with Kala-Azar fever (Conrad, 2021). In 1921the genus Phlebotomus of sandflies were confirmed as vector (Benallal et al., 2022). Due to challenges in vector control and absence of vaccines treatment primarily relies on Chemotherapy. Morphology and Nomenclature of Leishmania are mentioned in Fig. 1 and Fig. 2.

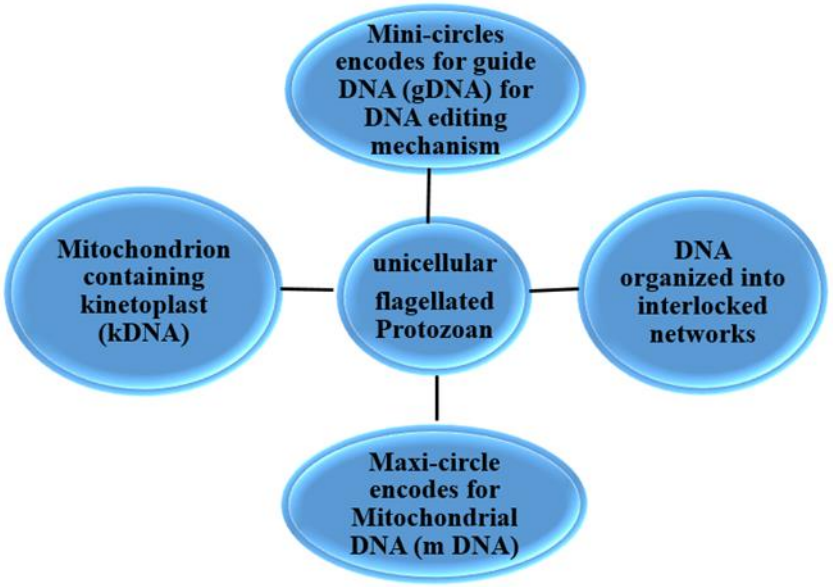

Fig. 1 Morphology of Leishmania

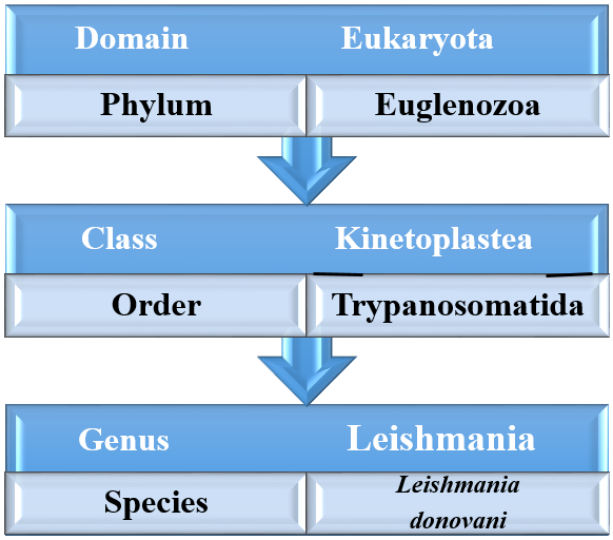

Fig. 2 Nomenclature of Leishmania

Types of Leishmaniasis

Leishmaniasis has been classified in three major forms traditionally on the basis of clinical symptoms.

1. Cutaneous Leishmaniasis (CL):

"Most common form of Leishmaniasis is cutaneous Leishmaniasis which presents as self-healing skin ulcer andoften called Oriental sore (Balahbib et al., 2023)".
It starts as a lesion at the sandfly bite site healing within months but leaving scars (Man et al., 2022). The incubation period ranges from days to months (Aoun et al., 2020). However, healing occurs as immune cells isolate the infection causing tissue necrosis and granuloma formation (Gurel et al., 2020).

Clinical Signs

Skin ulcers, cutaneous nodules and plaques, Satellite lesions

Treatment

- Physical methods: cryotherapy (liquid nitrogen)
- Application of local heat (e.g. infrared lamp)

2. Mucocutaneous Leishmaniasis (MCL):

"Mucocutaneous Leishmaniasis also known as espundia causes severe damage to naso-oral and pharyngeal regions are leading to disfiguring facial lesions".
It is typically transmitted zoonotically between rodents and mammals by Lutzomyia sandflies (Baneth et al., 2021).

Clinical Signs

An initial skin lesion that gradually heals on its own, chronic ulcers may develop months or years later affecting the skin, mouth and nose while causing damage to the underlying tissues. These lesions typically contain very few parasites.

Treatment

- Meglumine antimonate (20 mg Sb/kg/day for 30 days)
- Amphotericin B (Fungizone)

3. Visceral Leishmaniasis (VL) or Kala-azar (KA)

"Visceral Leishmaniasis also known as Kala-Azar fever. It is the most severe form of Leishmaniasis often fatal if left untreated (Pasha et al., 2022)". It involves parasite invasion of macrophages by affecting organs like the spleen, liver and bone marrow (Poulaki et al., 2021).

Clinical signs:

It is characterized by prolonged fever, splenomegaly, hepatomegaly, substantial weight loss, progressive anemia, pancytopenia, and hyperglobulimia.

Treatment

- Glucantime (meglumine antimonate, 85 mg Sb/ml)
- Pentostam (sodium stibogluconate, 100 mg Sb/ml)

Life Cycle of Leishmania donovani:

The disease is anthroponotic (means humans are the primary reservoirs and it spreads mainly through the bites of female sandflies, particularly Phlebotomus argentipes (Fig. 04) (Secundino et al., 2022; Tripathi & Nailwal, 2021).
Leishmania parasites have two distinct forms (Fig. 03):
1. **Promastigotes:** These are extracellular, elongated, flagellated and motile measuring 2µm × 2-20 µm (Kitoyi, 2023). This form is found in the sandfly and in laboratory cultures.

2. **Amastigotes:** These are intracellular, round or oval, non-motile and lack flagella with a size range of 2-5 µm (Beyaz, 2022). They live and multiply within the phagolysosomes of macrophages in the host's reticuloendothelial system (Paul et al., 2022).

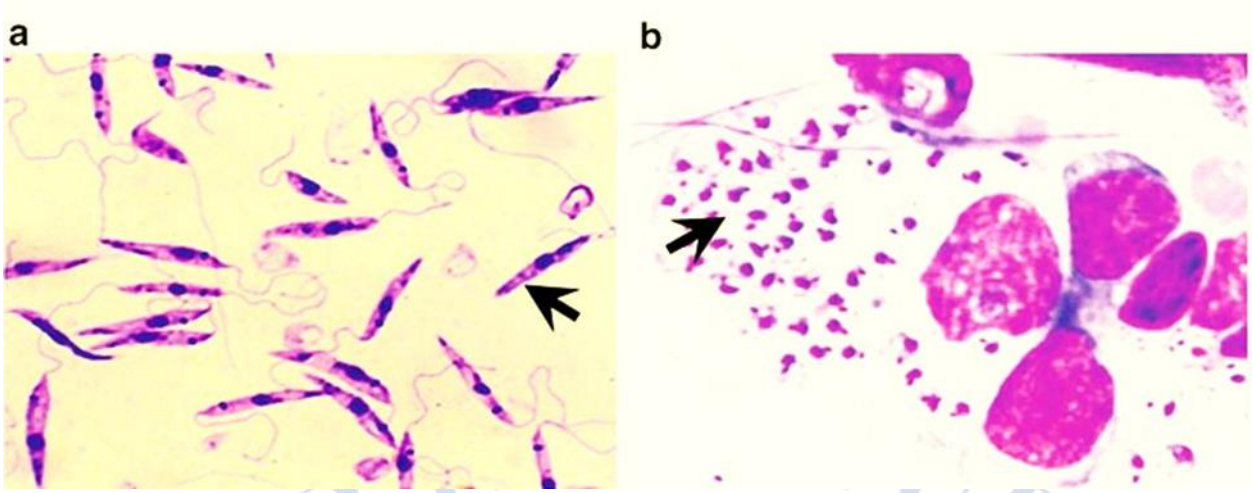

Fig.3 The Leishmania parasite exists in two distinct stages: (a) The promastigote stage, characterized by its motility and extracellular nature, each possessing a flagellum. (b) The amastigote stage, which is intracellular and non-motile, appearing as small dots under a microscope. These are visible in a Giemsa-stained dab smear from the spleen of a golden hamster infected with Leishmania donovani.

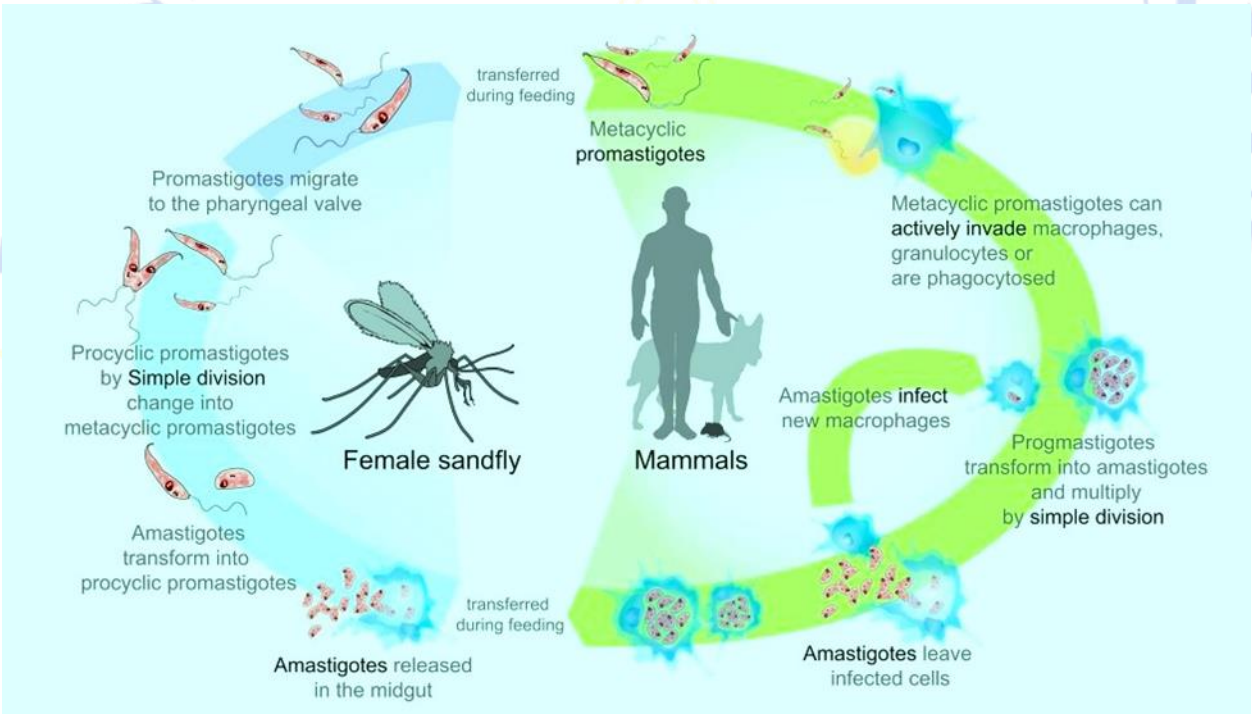

Fig. 4 The life cycle of the Leishmania parasite alternates between the sandfly vector and the human host.

Leishmaniasis's Vector

Sandfly Transmission: Leishmaniasis spread to dogs via bites of phlebotomine sandflies. Sandflies prefer short- haired breeds and bite hairless areas like ear pinna, nose and inguinal regions (Fig. 5).

Fig. 5 Sandfly, the vector host of Leishmania parasite

Diagnosis Methods

i) **PCR Testing:** PCR offers rapid detection (within 24 hours) and high sensitivity but is costly and requires specialized equipment and trained personnel making it less feasible in endemic regions.

ii) **LAMP Assays:** LAMP assays simplify diagnosis with constant-temperature DNA amplification (600c) and lyophilized reagents. It is easy to visualize reducing coats and complexity.

Control Strategies of the Disease

- Early diagnosis and timely treatment reduce morbidity, prevent mortality and help control disease transmission especially in anthroponotic areas.
- Treating cases of Leishmaniasis is crucial it serves as long term reservoirs of diseases.
- Residual insecticide spraying is effective for sandflies.
- In areas where sandflies bite at night insectides is treated bednets have successfully reduced Leishmaniasis cases.

Immunology of Leishmania:

The immune response against Leishmania involves lymphocytes, macrophages and antibodies while antibodies produced (IgG) are non-protective (Almeida et al., 2023; Lodi et al., 2024). Leishmaniasis shows elevated Humoral responses with hyper-gammaglobulinemia aiding diagnosis (Baxarias et al., 2023). Th1 cells mediate resistance through cytokines like IFN-Ɣ although Th2 cells exacerbate disease with IL-4 and IL-10 (Bamigbola & Ali, 2022; Flórez et al., 2021). Similar Th1/Th2 cytokine dynamics are observed during Leishmaniasis in diseased species (Fig. 6) (Samant et al., 2021).

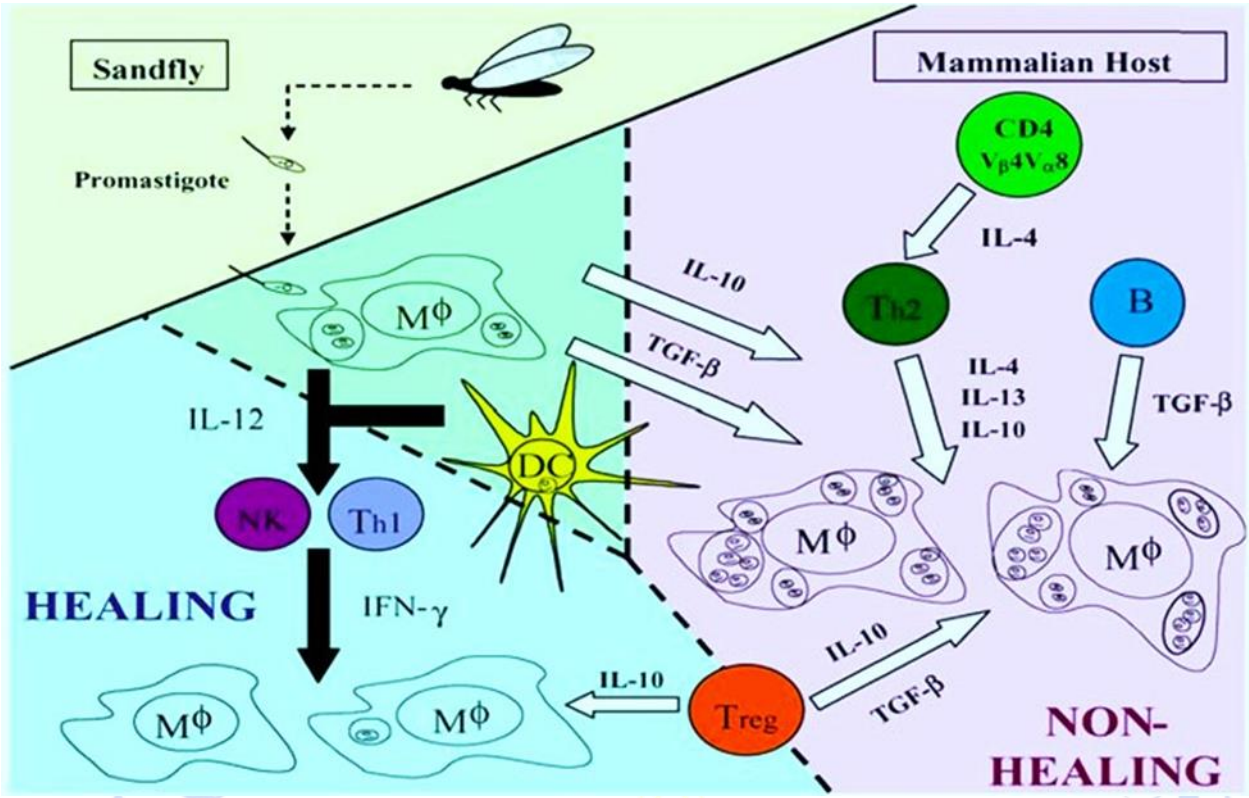

Fig. 6 Immune Response to Leishmania

When an infected sandfly takes a blood meal, it transmits metacyclic promastigotes into the vertebrate host. These transform into amastigotes upon entering macrophages and dendritic cells. The infected cells produce IL-12, which activates natural killer (NK) cells and promotes the differentiation of CD4+ T-helper 1 (Th1) cells, leading to the release of interferon-gamma (IFN-γ). IFN-γ stimulates macrophages to express inducible nitric oxide synthase (iNOS), resulting in nitric oxide (NO) production that kills the parasite and supports a healing response.

Role of B Cells and Immunoglobulin

Polyclonal B cells activation occurs in visceral Leishmaniasis. In Cutaneous Leishmaniasis B cells and antibodies can enhance susceptibility with IgG coated Amastigotes promoting parasite growth via IL-10 (Dermicik et al., 2021).

Role of T Lymphocytes

In Leishmaniasis immunity is mainly driven by T lymphocytes (Saha & Silvestre, 2021). Early studies with T cell depleted and nude BALB/c mice has highlighted the role of T cells in protecting against L.donovani infection (Zayats, 2023; Dong et al., 2021). The both CD+4 and CD+8 T cells are essentials for immunity (Badhwar et al., 2024; Osorio et al., 2023).

Role of Cytokines

T lymphocytes contributes to immunity against L.donovani by producing cytokines like IFN-Ɣ and IL-2 are essential for granuloma formation and infection control (Santi et al., 2018). While IFN-Ɣ is crucial for protection IL-4 and IL-5 which are not involved and IL-10 is linked to disease progression with its role in susceptibility to Leishmania infection (Fig. 7) (Rodrigues et al., 2023).

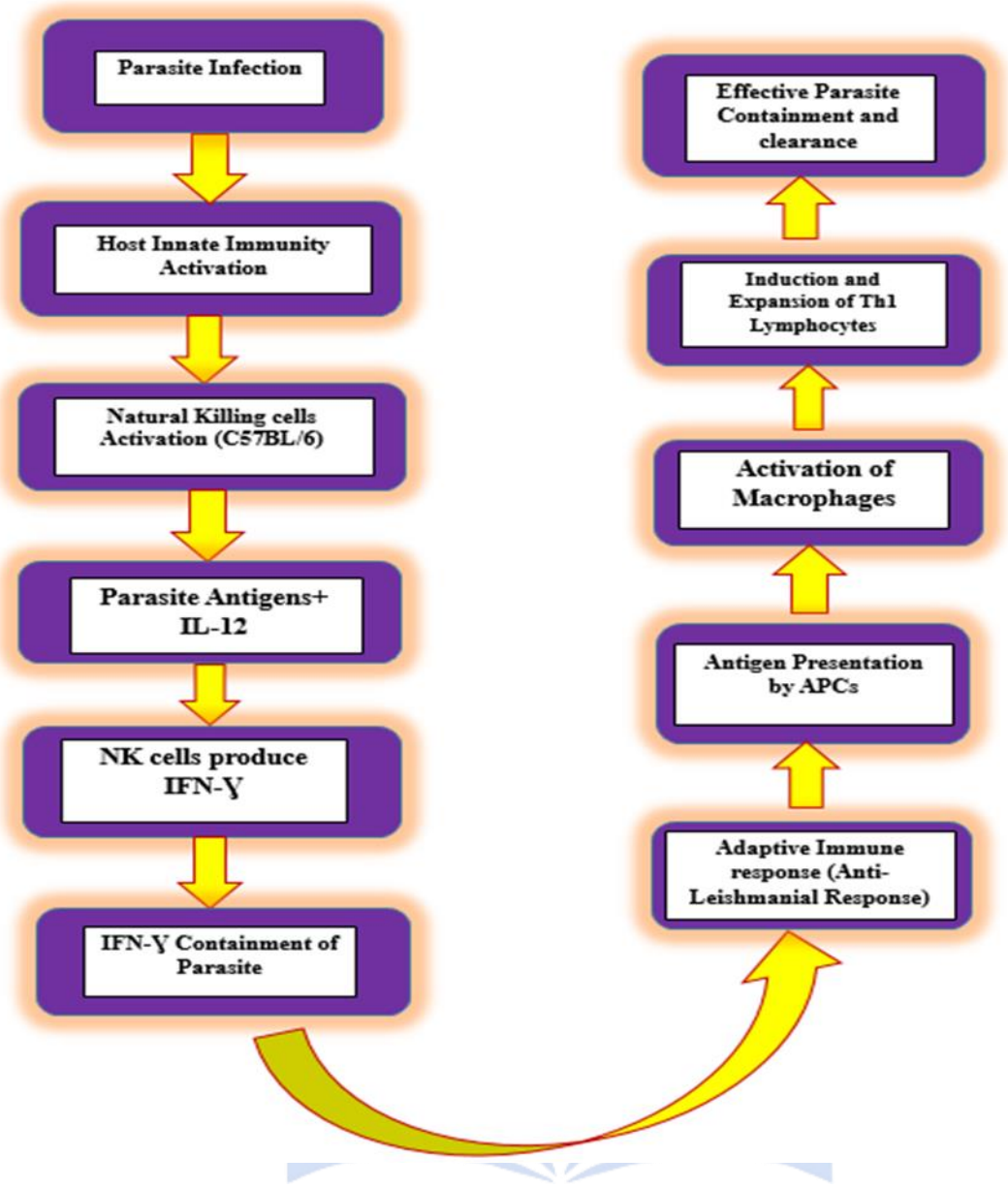

Fig. 7 Host Immune Response to Leishmania Infection

Epidemic of Leishmania (Invade and Survival Strategy of Leishmania)

- **Leishmania Life cycle:** The parasite is transmitted between the sandfly vector and the mammalian host. Within the sand fly after ingesting infected blood the parasite transforms from the amastigote form into the promastigote form (Omondi et al., 2022).

- **Promastigotes Development:** Promastigotes divide in the midgut and undergoes metacyclogenesis into infective metacyclic promastigote which move to foregut suspended in saliva (Seow, 2021).

- **Role of Saliva:** Sand fly saliva aids parasite survival and promotes inflammation, immune suppression (IL-4 dependent) and disease progression through components like Maxadilan (Schneider et al., 2021).

- **Parasite Infection in Mammals:** Leishmania infects host immune cells (macrophages, dendritic cells) through various receptors (Kupani et al., 2021). The parasite is engulfed forming a parasitophorous Vacuole (PV) where it transforms into Amastigotes. Amastigotes lack LPG containing proteophosphoglycan acid phosphatse and GIPLs (EI Saftawy et al., 2021).

Vaccination for Leishmaniasis:

Some approved vaccines for animal Leishmaniasis are as follows:

- Letifend was licensed in Europe in February 2016, is a veterinary vaccine used in dogs.
- Leishmune was the first licensed vaccine for CanL, registered in Brazil in 2004.
- Leish-Tec was licensed in Brazil in 2007.
- CaniLeish was released in Europe in 2011.
- LetiFend was licensed in Europe in February 2016.

Specimen (Rabbit):

Recent investigations following a Leishmaniasis outbreak in Southwest Madrid with over 446 reported human cases have identified the Lberian hare (Lepus granatensis) as a new reservoir for the disease (Cardoso et al., 2021; Santos, 2020). PCR and indirect immunofluorescence antibody tests confirmed infection with L. infantum in Hares and xenodiagnostic studies showed their ability to transmit parasite to sandflies (Phlebotomus perniciosus). This evidence suggests that hares majorly contribute to this epidemic outbreak (OMBULA, 2024).

Study on Hares:

A separate study on 94 hares across Spain found a 43.6% prevalence of L. infantum infection is indicating the significance of Lberian hares in Leishmaniasis transmission (Tsakmakidis et al., 2024). Additionally in the Madrid outbreak area 46% seroprevalance was found in Rabbits indicates that they play a role in maintaining the infection (Larena, 2022). Further research in high density rabbit area in Madrid using both serology and PCR based methods assessed the potential risk of Rabbits as a source of infection (Calvete et al., 2022).

Symptoms of Leishmaniasis in Rabbits::

Leishmaniasis in rabbits particularly caused by Leishmania infantum cause a range of symptoms.
Such symptoms are as follows:

1. Skin Lesions (ulcer, sores, crusty lesions around eyes, face and legs)
2. Hair Loss (Focal and general thinning of fur especially around lesions)
3. Loss of Appetite (loss of weight, Lethargy and reduced activity)
4. Lymphadenopathy (swollen lymph nodes in head and cervical regions)
5. Anemia (Drop in Red Blood Cells leads to pale mucous membranes)
6. Pyrexia (mild to moderate fever in body)
7. Gastrointestinal infection (Diarrhea and bloating)
8. Respiratory infections (Respiratory distress, coughing and Nasal discharge)
9. Immunosuppression (Increased susceptibility to susceptibility to secondary infections due to weak immune system) (Fig. 8)

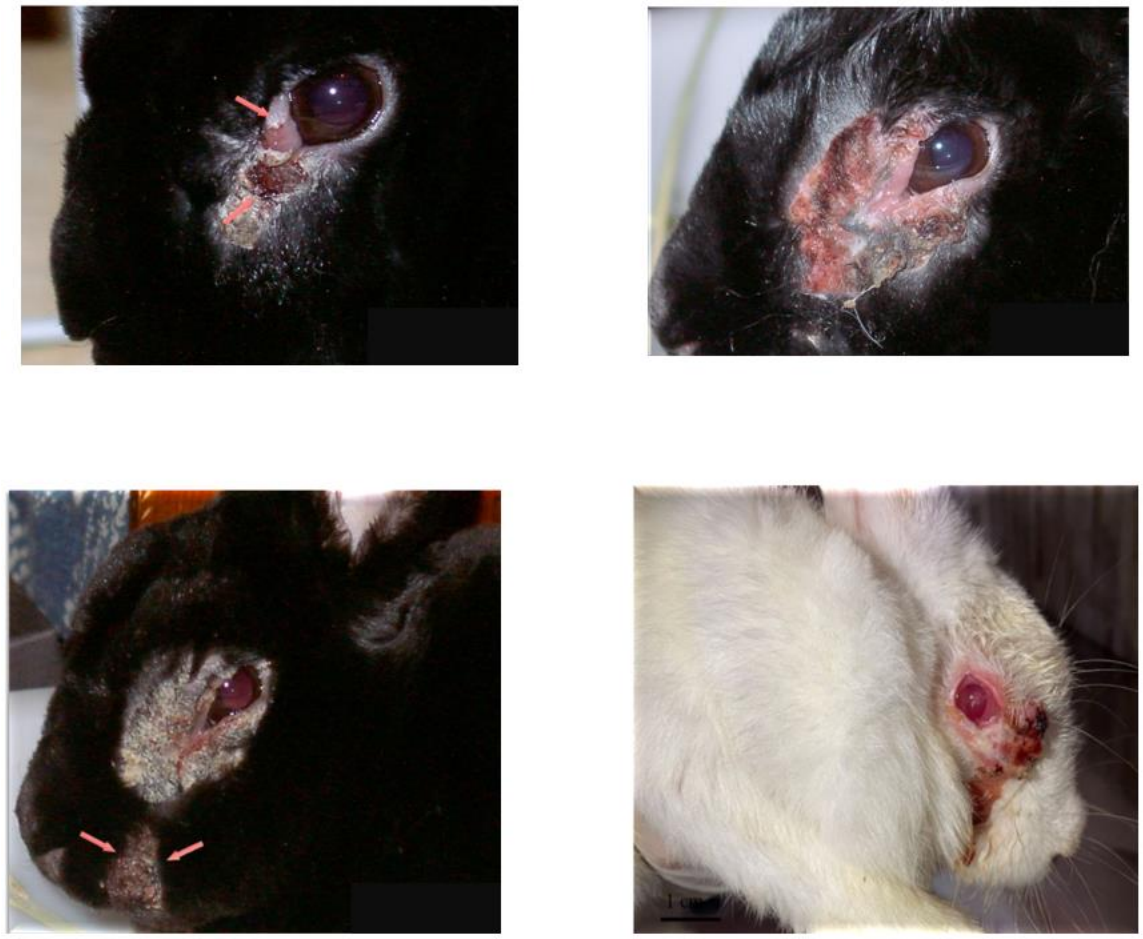

Fig.8 Symptoms of Leishmaniasis in Rabbits

Preventive Measures for Leishmaniasis in rabbits:

- control of hare and rabbit populations in the affected area
- monitoring program at risk points was carried out through the collection of specimens
- Sanitation measures have been implemented at risk points and collection of abandoned animals was also enforced.

Adjuvants for Leishmaniasis in Rabbits:

Adjuvants: "Adjuvants are the substances that are added to vaccines to enhance the immune response to an antigens (Fig. 9) (Zhao et al., 2023)". The Adjuvants used in Leishmaniasis research in Rabbits are essentials for improving immune response and developing effective vaccines (Table .1) (Moni et al., 2023; Das & Ali, 2021). These adjuvants help in enhancing both Humoral and Cellular immunity with a particular focus on stimulating Th1 responses which are essential for controlling Leishmania infections (Ikeogu et al., 2020). The choice of adjuvants depend on the nature of Vaccines and the type of immune response desired and mainly the characteristics of studied Rabbit model (Gong et al., 2020).

Using immunologic adjuvants in candidate vaccines for VL offers several advantages including boosting immunogenicity, are mentioned in table 1 (Silva et al., 2023). It's effectiveness promotes a Th-1 type T cell response, reducing the required number of doses sustaining the activation of specific effectors CD4+ and CD8+ T cells and increasing the duration and strength of neutralizing antibody responses (Ikeogu et al., 2020).

Table 1.. List of particulate Adjuvants used with Vaccine against various Leishmaniasis

Adjuvant	Vaccines	Against infection	References
Aluminium Hydroxide	Alum form a short depot at site of injection and slowly release vaccine antigens to immune system	Visceral Leishmania	(Cardoso et al., 2021; Santos, 2020)
Alum-BCG-imiquimod	White gel like substance, insoluble in water	Mucocutaneous Leishmania	(Larena, 2022)
Liposome	Able to encapsulate and deliver antigens to specific immune T cells	Cutaneous Leishmania	(Calvete et al., 2022)
Montanide	Induced both Th1 type cellular and humoral response	Leishmania donovani	(Tsakmakidis et al., 2024)
Freunds	Adjuvant with prolonged antigens persistence that activates phagocytosis	Leishmania infantum	(OMBULA, 2024)

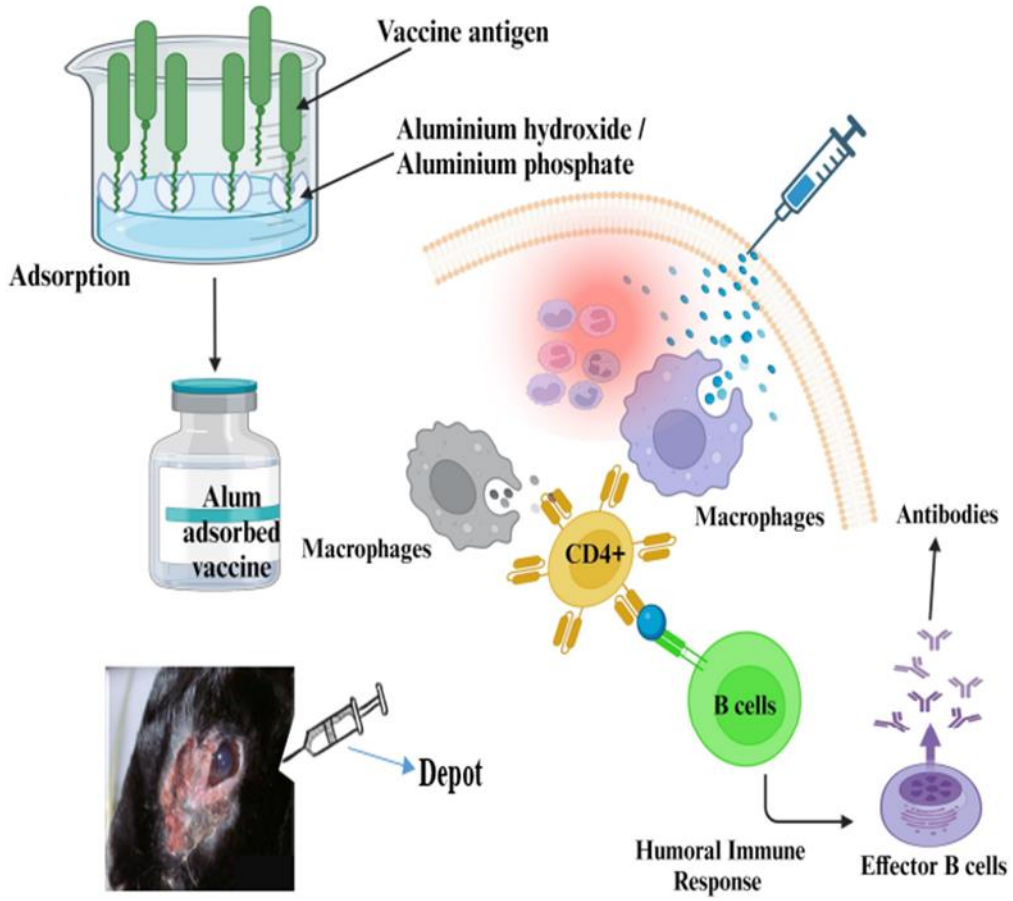

Fig. 9 The mechanistic approach of aluminum salts as vaccine adjuvants

Relationship of Adjuvants with Antibiotics:

Ramon has demonstrated that are adding substances like Alum to inactivated Diphtheria toxin enhanced local inflammation and antibody formation to establish the role of adjuvant in boosting vaccine efficacy (Gogoi et al., 2024). Glenny's in 1926 findings highlighted Alum's importance to improve immunogenicity leading to vaccines incorporation adjuvants in stimulating targeted immune responses. Adjuvants not only enhance antigens presentation and CTL responses but also support simplified immunization protocols that reduce the costs and benefit the immunocompromised individuals (Diaz-Dinamarca et al., 2022).

Novel Antigens in Leishmaniasis:

Developing Vaccines against Leishmaniasis involves identifying and utilization Novel antigens that can elicit a protective immune response (Ayala et al., 2024). Here are some Novel Antigens in Leishmaniasis. Protein based antigens such as KMP-11, GP63 and heat shock proteins show promise for stimulating Th1-biased immunity while Histone derived proteins are strong inducers of CD+8 T cell responses. Glycan-based molecules like lipophosphoglycan (LPG) and glycosylphosphatidylinositols (GPIs) are potential candidates due to their immune-modulating properties. Parasite-specific proteins includingA2 proteins and LACK have demonstrated protective efficacy in animal models. In recent researches the combining of all these antigens with adjuvants targeting the intracellular amastigote stage and leveraging omics technologies to identify and develop effective vaccines (Kaye et al., 2021).

Conclusion

In conclusion, Leishmaniasis is a significant parasitic disease with diverse clinical forms and a substantial global health impact. Its prevalence in both humans and animals necessitates innovative strategies for prevention and treatment. Rabbits are increasingly recognized as reservoirs for Leishmania infantum which underline the urgent need for effective veterinary vaccines to mitigate zoonotic transmission risks. Advancements in vaccine development have focused on novel antigens and adjuvants which are crucial in stimulating robust immune responses. Protein-based antigens like KMP-11 and GP63, as well as glycan-based molecules are lipophosphoglycan that show promise in producing protective immunity. These antigens are combined with adjuvants like alum toenhance antigen presentation with stimulating Th1 and CD8+ T-cell responses that are vital for controlling Leishmania infections. Adjuvants also improve vaccine immunogenicity, reduce dosing requirements, and support immunity in compromised hosts by making them pivotal in vaccine efficacy.

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