Review protocol

Welfare implications of Complete Freund's Adjuvant (CFA) use in laboratory animal immunization and antibody-production models: a scoping review

Administrative Information

PRISMA-P Topic 1: Title

Topic 1a: Identification

The impact of Complete Freund´s Adjuvant on the welfare of laboratory mice, rats, and rabbits used for antibody production: a scoping review

Topic 1b: Update

This protocol is not an update of a previously conducted review. It pertains to an original scoping review.

PRISMA-P Topic 2: Registration

This protocol will be submitted to the Systematic Reviews for Animals and Food (SYREAF) - http://www.syreaf.org/. This protocol follows the PRISMA-P guidelines (Moher et al., 2015); some adaptations must be made, given that the original PRISMA-P guidelines were designed for systematic reviews, whilst this protocol outlines the plan for a scoping review.

The review will be reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines (Tricco et al., 2018).

PRISMA-P Topic 3: Authors

Topic 3a: Contact

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Topic 3b: Contributions

Contribution	**Authors**
Concept idea	OM, CB, BT, LPC, VH
Drafting protocol	VH, LPC
Reviewing protocol	CB, LPC, VH, AV, OM, BT
Defining eligibility criteria	CB, LPC, VH
Search strategy	VH, LPC (input from all authors)
Title and abstract screening	VH, AV
Full-text screening	AV, VH, LPC
Data extraction	VH, AV, LPC
Data analysis and synthesis of results	VH, AV, LPC
Drafting paper	VH, AV, CB, LPC
Reviewing paper	All

PRISMA-P Topic 4: Amendments

None to be reported.

PRISMA-P Topic 5: Support

Topic 5a: Sources

This review was performed in the frame of the core finance contract between the Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern and the Swiss Federal Food Safety and Veterinary Office.

Topic 5b: Sponsor

This review received funding from the Swiss Federal Food Safety and Veterinary Office.

Topic 5c: Role of sponsor or funder

The sponsor/funder was involved in the development of the study concept. The study aims to provide updated evidence on the animal welfare implications of complete Freund's adjuvant in laboratory animals (mice, rats, and rabbits) and to supplement existing knowledge with information from recent scientific literature.

Introduction

PRISMA-P Topic 6: Rationale

Adjuvants play a key role in experimental immunology by enhancing and directing immune responses to antigens. Among the most potent adjuvants is Complete Freund's Adjuvant (CFA): a water-in-oil (w/o) emulsion containing heat-killed *Mycobacterium tuberculosis*. This formulation combines the immunomodulatory effects of mycobacterial components with the sustained antigen release afforded by the w/o emulsion depot effect (Cox & Coulter, 1997). Given these strong immunogenic properties, CFA continues to be used in animal models and antibody production to induce high-titer, high-affinity immune responses (Stills, 2005), often based on historical precedent rather than explicit comparative evaluation against newer adjuvant systems. However, the same mechanisms that make CFA so effective also give rise to significant animal welfare concerns. CFA can cause long-lasting local inflammation, accompanied by pain and ulceration at the injection site (Billiau & Matthys, 2001; Bastola et al., 2017). Its reactogenic nature can lead to necrotic tissue damage, sterile abscesses, and systemic granulomatous lesions in various organs (Broderson, 1989; Leenaars et al., 1998; Stills & Bailey, 1991; Apostólico et al., 2016). The severity and persistence of these adverse effects are strongly influenced by species, injection route, dose, and frequency, and may exceed moderate distress under certain experimental conditions. Reflecting these risks, the Swiss Federal Food Safety and Veterinary Office (FSVO) classifies immunizations with CFA in Switzerland as a severity degree 2 procedure, meaning it can cause short-term moderate or medium-term pain or distress (FSVO, 2018), while requiring case-by-case justification and careful weighing of interest assessment.

Despite these challenges, CFA remains in use across various preclinical research settings. Modern guidelines highlight the need for restraint and refinement in its use. The American *NIH Guidelines for the Use of Adjuvants in Research* (2025) emphasize that CFA use must be scientifically justified, limited, and accompanied by appropriate analgesic measures. The Swiss Animal Welfare Act (SR 455) requires that procedures involving pain or distress must be justified and limited to the indispensable minimum (Art. 17, Art. 20 AWA). The FSVO's Technical Information 3.04 on 'Proper and Animal Welfare-Compliant Antibody Production in Rabbits, Chickens, and Laboratory Rodents' further delineates limitations regarding injection routes, booster use, and indications for CFA administration. This emphasizes the expectation that CFA should not be used where less harmful alternatives can achieve comparable scientific outcomes.

Balancing the advantages of CFA as an adjuvant with the ethical imperative of animal welfare remains a critical challenge. In light of evolving regulatory requirements in Switzerland and the documented availability of alternative adjuvants with differing safety profiles, a structured synthesis of existing evidence on CFA-associated animal welfare effects and their justification is currently not available. A comprehensive understanding of its welfare implications is essential to inform evidence-based recommendations related to the use of CFA in scientific experiments, promote refinement in experimental practice, and guide future policy development.

PRISMA-P Topic 7: Objectives

This review aims to:

(i) Document and characterize the existing literature describing the welfare impacts associated with the use of CFA in mice, rats, and rabbits;

(ii) Summarize and categorize the reported welfare effects (namely reported side effects), including their severity and the methods or indicators used to assess them; and
(iii) Identify and summarize any available evidence on potential alternatives to CFA used for immunization or antibody production in laboratory animals.

Methods

PRISMA-P Topic 8: Eligibility Criteria

The objectives can be summarized using a PICo (**P**roblem, **I**nterest, **Co**ntext) format with the following question: What are the impacts of the use of CFA on the welfare of laboratory animals?

Table 1. PICo structure

P	I	Co
Population/Patient/Problem	**Interest**	**Context**
Laboratory animals (mice, rats, rabbits)	(Impact on) Animal welfare	Use of CFA

A description of the inclusion/exclusion criteria can be found below:

- **Time:** 2015 – present (*The time frame was chosen to capture contemporary welfare evidence together with current standards in husbandry, veterinary care, and adjuvant formulation).*
- **Language:** English.
- **Type of publication:** Peer-reviewed.
- **Type of study:** only original research will be included. Reviews will be excluded; however, reviews will be screened in a search verification step to identify potential literature of interest not identified in our own electronic search. Given the nature of the population of interest, only experimental studies will be considered.
- **Welfare:** reported side effects related to the use of CFA will be included in this review. Events will be considered regardless of whether they produce observable disturbance in animals (differentiating between varying levels of animal impact falls outside the scope of this analysis).
- **Laboratory animals:** Mice, rats, rabbits.
- **CFA:** Administration of **CFA** (Complete Freund's Adjuvant and not Incomplete Freund's Adjuvant) for immunization or antibody production (including primary/booster injections). *Explicitly excluded: arthritis/pain-induction models*.

PRISMA-P Topic 9: Information Sources

Electronic searches will be performed in Pubmed and Embase. These databases offer good coverage of health and natural sciences, which overlap with the topic under review.

As previously mentioned, reviews on the topic will be used to identify additional references.

PRISMA-P Topic 10: Search Strategy

The search string will include three main groups of search terms: Laboratory animals (mice, rats, and rabbits), use-case: immunization/antibody production, and the use of CFA. The Boolean operator "AND" will be used between the two groups. Wildcards will be used according to their availability in each database.

Following several rounds of testing and finetuning, which included consultation with experts in the field, the following search strings will be used:

Pubmed

(("mouse"[Title/Abstract] OR "mice"[Title/Abstract] OR "rat"[Title/Abstract] OR "rats"[Title/Abstract] OR "rabbit*"[Title/Abstract] OR "murine"[Title/Abstract] OR "murines"[Title/Abstract] OR "Murinae"[Title/Abstract] OR "Murinae"[MeSH Terms] OR "Rabbits"[MeSH Terms]) AND ("Immunization"[Title/Abstract] OR "immunisation"[Title/Abstract] OR "vaccination"[Title/Abstract] OR "vaccine*"[Title/Abstract] OR "antibody production"[Title/Abstract] OR "antibody generation"[Title/Abstract] OR "antibody formation"[Title/Abstract] OR "antibody response"[Title/Abstract] OR "antibody titer"[Title/Abstract] OR "antibody level*"[Title/Abstract] OR "neutralizing antibod*"[Title/Abstract] OR "seroconversion"[Title/Abstract] OR "polyclonal"[All Fields] OR "monoclonal"[All Fields] OR "immunogenicity, vaccine"[MeSH Terms] OR "antibody formation"[MeSH Terms] OR "Immunization"[MeSH Terms] OR "vaccine development"[MeSH Terms]) AND ("Complete Freund's Adjuvant"[Title/Abstract] OR "Freund's complete adjuvant"[Title/Abstract] OR "CFA"[Title/Abstract] OR "Freund's Adjuvant"[MeSH Terms])) AND (2015:2025[pdat])

Embase

('freund adjuvant':de OR 'complete freund adjuvant':tn,ti,ab OR 'complete freund type adjuvant':tn,ti,ab OR 'freund complete adjuvant':tn,ti,ab) AND ('mouse':de OR 'rat':de OR 'leporidae':de OR 'murine':ab,ti) AND ('immunization':ab,ti OR 'antibody production':ab,ti OR 'antibody response':ab,ti OR 'antibody titer':ab,ti OR 'neutralizing antibody':ab,ti OR 'seroconversion':ab,ti OR 'vaccine immunogenicity':ab,ti OR 'monoclonal antibody':ab,ti OR 'polyclonal antibody':ab,ti OR 'immunization':de OR 'antibody production':de OR 'vaccine immunogenicity':de) AND [2015-2025]/py

PRISMA-P Topic 11: Study Records

Topic 11a: Data management

References will be imported to the software Rayyan, where automatic and manual deduplication of references will be performed. This software will also be used to facilitate the screening process. ChatGPT (OpenAI, GPT-5 model) will be used to facilitate the screening process and data extraction, as described in Topics 11b and 11c. All prompts and ChatGPT outputs will be

archived and version-controlled (including ChatGPT iteration and functionality – i.e., ChatGTP-5 "deep thinking").

Topic 11b: Selection process

The selection process will be conducted by reviewers with the support of ChatGPT. ChatGPT will assist during the title/abstract and full-text screening phases to enhance consistency, transparency, and documentation of the selection procedure. A structured screening template will be implemented to ensure the uniform application of predefined inclusion and exclusion criteria (i.e., studies using CFA in rodent immunization or antibody-production models, excluding those involving arthritis or pain models).

The reviewers will manually verify all output generated by ChatGPT before a given publication is included or excluded. ChatGPT will serve solely as a tool to facilitate text identification and organization and will not make independent inclusion or exclusion decisions.

The selection process will be conducted in two phases:

a) **Title and abstract screening:** A calibration exercise will be conducted using 20 randomly selected articles screened by all reviewers, in parallel, manually and with ChatGPT. Inter-reviewer agreement will be assessed, and the process refined as needed. Only studies involving mice, rats, or rabbits will be retained; studies using other rodent species will be excluded at screening. Once a high level of agreement is achieved, each title and abstract will be screened by one reviewer (manually and with ChatGPT), applying the eligibility criteria defined in *PRISMA-P Topic 8: Eligibility Criteria*. A high-sensitivity approach will be adopted; in cases of uncertainty, the study will be retained for full-text review.

Prompt used for ChatGPT during title and abstract screening:

Please determine whether the study meets the following criteria based on its title and abstract:
1. Does the study describe an **immunization or antibody production model** (not an arthritis or pain model)?
2. Does the study involve **rodent species or leporidae** (e.g., mice, rats, or rabbits)?

If both criteria are met, classify the study as **"Include for full-text review."**
If either criterion is unclear or cannot be determined, flag the study as **"Uncertain – check manually."**

b) **Full-text screening:** The full-text screening phase will follow a similar approach. A calibration exercise will first be conducted using five randomly selected articles reviewed by all participating reviewers to ensure consistency. Following calibration, each full text will be screened by one reviewer, both manually and with ChatGPT, using the eligibility criteria outlined in *PRISMA-P Topic 8*. In cases of uncertainty, the study will be discussed among the reviewers, and a consensus decision will be reached. Reasons for exclusion will be documented.

Prompt used for ChatGPT during full-text screening:

Please screen the following study (abstract or full text) according to the criteria below and summarize the results in a table with the columns *Item*, *Answer*, and *Supporting Text (verbatim quotes)*.

Screening criteria:
1. **CFA use:** Does the paper explicitly describe the use of Complete Freund's Adjuvant (CFA)?
2. **Experimental model:** Is the context immunization or antibody production (exclude arthritis or pain models)?

3. **Animal species:** Does the study use rodents or leporidae (mouse, rat, other rodent species or rabbits)?
4. **CFA-related effects:** Are any CFA-related welfare effects reported (e.g., local inflammation, granuloma, systemic effects or any other clinical symptom or effect on animal health and/or wellbeing)?
5. Include all relevant text excerpts that justify your answers. If any information is ambiguous or missing, flag it as **"Check manually"** and note the uncertainty.

The inclusion decision is made by the reviewers.

Topic 11c: Data collection process

A data extraction form will be created in Excel. The articles included following the full text screening will be included in the data extraction step.

Data extraction will be conducted using ChatGPT and independently verified by a reviewer for each included manuscript. ChatGPT will assist in the data collection process by identifying and organizing relevant information from each study based on predefined data fields. A standardized prompt template will ensure consistency and transparency. All outputs will be manually reviewed for accuracy and completeness, and the presence or absence of data items will be verified directly in the original papers to avoid misinformation due to AI hallucinations. ChatGPT will be used solely to facilitate text extraction and organization, without independently interpreting or coding findings.

PRISMA-P Topic 12: Data Items

The following data items will be extracted from each individual study:

- Study identifiers: Author, Affiliation, year, journal, country, funding, conflicts.
- Animal characteristics: Species, strain, sex, age
- CFA: Antigen, CFA concentration, volume, route (intradermal, subcutaneous, footpad, etc.), IF injection THEN injection site (tail base, flank, footpad, etc.), number and timing of injections/boosters.
- If available, comparator (alternative) details: Adjuvant type, dose, route; or none.
- Welfare outcomes: As noted under primary outcomes, include definitions/scales used; timepoints, adverse effects; number of animals with adverse effects and total number of animals; or any other clinical symptom or effect on animal health and/or wellbeing.

As described above in "Topic 11c: Data collection process" ChatGPT will assist in the data collection process. The following prompt will be used.

Standardized data extraction prompt (ChatGPT)

Please extract the following information from the provided study (abstract or full text). Use verbatim sentences wherever possible and summarize only when necessary for clarity. Present the results in a table with the columns *Data Item*, *Extracted Information*, and *Supporting Text (quotes)*.

1. **Study identifiers:** Author(s), year, journal, country, funding source, and conflict of interest statement.
2. **Animal characteristics:** Species and strain of rodents.

3. **CFA characteristics:** Antigen used, CFA concentration, injected volume, route of administration (e.g., intradermal, subcutaneous, footpad), injection site (e.g., tail base, flank, footpad), and number and timing of injections or boosters.
4. **Comparator details (if applicable):** Adjuvant type, dose, route, or indication that no comparator was used.
5. **Welfare outcomes:** Reported indicators of welfare or adverse effects (as defined under primary outcomes), including scales or definitions used, observation timepoints, number of animals affected, and total number of animals assessed.

After extraction, provide a **summary paragraph** noting any ambiguities or missing information.

PRISMA-P Topic 13: Outcomes and prioritization

Outcomes related to animal welfare will be recorded for each study, as described above. Outcomes will not be ranked or prioritized; all welfare-related outcomes reported in the studies will be summarized descriptively.

PRISMA-P Topic 14: Risk of bias in individual studies

This step will not be conducted. This is not a mandatory step for scoping reviews, and given the expected heterogeneity of the studies and different outcomes reported, we will not assess the impact of bias on the outcomes.

PRISMA-P Topic 15: Data synthesis (15a to 15d)

Descriptive statistics will be produced for every data variable extracted from the included studies. Data will be summarized using frequencies, percentages, and cross-tabulations for variables (i.e., route of administration, animal species, etc.). Thematic grouping will be applied to qualitative welfare outcomes.

PRISMA-P Topic 16: Meta-bias(es)

Not applicable.

PRISMA-P Topic 17: Confidence in cumulative evidence

Not applicable.

References

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