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Development and In Vivo Evaluation of Recombinant Multi-Epitope Vaccine (ABOR) with Chitin Microparticles as Adjuvant Against Brucella Abortus

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Abstract | Immunization plans need to function more efficiently since human health as well as cattle face a substantial threat from brucellosis which is transmitted to people through Brucella spp. Additional risk-free options must be developed given that existing live-attenuated vaccines including RB51 display two fundamental constraints which are diagnostic interference and residual disease potency. Research and analysis created and evaluated a new ABOR recombinant multi-epitope polypeptide vaccine that utilized antigenic Brucella abortus protein sequences. The polypeptide emerged from Escherichia coli BL21 bacteria and the study explored its binding with chitin microparticles when used as an adjuvant. Research into the immunogenic properties of guinea pigs involved performing tests on their serum and cellular immune responses. The ABOR+chitin vaccination stimuli led to IgG increases equaling RB51 vaccination levels according to ELISA analysis. The real-time RT-PCR examination confirmed the development of a Th1 dominant cytokine profile together with elevated levels of IFN-γ and IL-12 and TNF-α and reduced levels of IL-4 and IL-10. The ABOR+chitin treatment demonstrated superior ability to generate antigen-specific T-cell responses in comparison to RB51 vaccination in lymphocyte proliferation tests. Investigations at the tissue level showed that the vaccine administration resulted in no hazardous effects to the liver structure thus confirming its safety profile. A combination of ABOR with chitin seems to trigger a considerable immune system response which indicates its potential to replace live-attenuated vaccines in preventing brucellosis.

Keywords | Brucellosis, *Brucella abortus*, Recombinant vaccine, Chitin adjuvant, Immune response, Cytokines, Th1 immunity, IgG, Vaccine safety, Antigen-specific T-cell response

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INTRODUCTION

Caused through Brucella spp., facultative intracellular pathogens mostly affecting cattle, brucellosis is a zoonotic bacterial illness, that may be acquired through people through contacting diseased animals or consuming contaminated dairy products (Khorramizadeh *et al.*, 2019). Reproductive failures, lower productivity, and trade restrictions within impacted areas cause the illness to have a major economic cost (Jacob and Curtiss, 2021). One for the most virulent species, Brucella abortus mostly affects cattle, and has been linked to serious human infections, thereby stressing the necessity for efficient management techniques (Zamri-Saad and Kamarudin, 2016). alongside live-attenuated vaccines as RB51, and S19 utilized extensively, the evolution for vaccinations has been very vital within brucellosis management. These vaccines have several important drawbacks, however, including residual virulence, possible toxicity to humans, and the capacity to cause miscarriage within pregnant animals, which calls for the hunt for safer, and more powerful substitutes) (Gheibi *et al.*, 2018; Kareem *et al.*, 2023; Aziz *et al.*, 2023). Brucella causes a complicated immunological response mostly including T-helper 1 (Th1) mediated immunity, which is necessary for bacterial clearance. Presenting Brucella antigens to lymphocytes, and inducing the release for pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-12 (IL-12), and interferon-gamma (IFN-γ), macrophages first line for protection, and help to activate cytotoxic T cells, and macrophages (Schurig *et al.*, 2002). Although the pathogen has developed strategies to avoid host defense through suppressing antigen presentation, and regulating cytokine release, hence causing persistent infections, this immune response is essential within the containment for Brucella infection (Babaoglu *et al.*, 2018).

Live-attenuated strains, including RB51, which lacks the O-polysaccharide component for the lipopolysaccharide (LPS), therefore minimising their interference alongside serological diagnostic tests (Puspitoyani *et al.*, 2020), current vaccination techniques for brucellosis mostly depend upon them. Though it has benefits, RB51 does not give 100% immunity against Brucella infection, and is not totally protective. Furthermore linked to miscarriages within pregnant cattle, and to ongoing infection within vaccinated animals is this agent (Yazdi *et al.*, 2009; Ali *et al.*, 2024; Al-Sailawi *et al.*, 2024; Mohsen *et al.*, 2024). Furthermore, studies indicate, that host genetics, and environmental circumstances affect the protective effectiveness for RB51, thereby stressing the necessity for different vaccination strategies (Dabral *et al.*, 2019). Subunit vaccines—which include immunogenic proteins or peptides, that induce specific immune responses while reducing side effects—have lately attracted most attention within the field for vaccine development. These vaccinations lower the potential for diagnostic interference, and eradicate bacterial persistence (Monreal *et al.*, 2004). Using multi-epitope chimeric proteins—which combine antigenic determinants coming from many Brucella proteins to improve immunogenicity—among the most exciting approaches Attractive possibilities for brucellosis prevention, studies show multi-component vaccinations produce stronger Th1-type response than single-component vaccines (Schurig *et al.*, 2002).

We produced a recombinant multi-epitope polypeptide vaccine (ABOR) which was made from several Brucella protein antigenic regions. The recombinant Escherichia coli (E. coli) BL21 (DE3) produced polypeptides received purification before being combined with chitin as an adjuvant material for immunological stimulation. The veterinary vaccination research model of choice in this study was the frequently validated animal specimen of guinea pigs which were used for ABOR immunogenicity tests. This study evaluated immune responses to antibodies and cytokine production (IL-12, IFN-γ, TNF-α, IL-10) together with T-cell activation to determine its potential as a secure and efficient alternative to live-attenuated vaccines for treating brucellosis.

MATERIALS AND METHODS

Preparation of ABOR Polypeptide

Defined by researchers ABOR polypeptide integrates five Brucella abortus protein-based antigenic domains which engineers them with GS linkers for protein connection. The synthesized coding sequence included sites for restriction enzymes to allow the sequence to be cloned into the pET22b expression vector obtained from GeneCust, Luxembourg S.A. The expression of the multi-epitope recombinant protein took place in Escherichia coli BL21 (DE3) strains after plasmid transformation resulted in the recombinant plasmid. Purification of recombinant ABOR polypeptide started with standard induction of protein expression then proceeded with chromatographic affinity separation. The lyophilization process to create a future-useable product occurred at −40°C along with full vacuum conditions for three hours under Pizarro-Cerda *et al.* (2000). The laboratories situated in Iraq served as the site to execute every experimental process.

Preparation of Chitin Microparticles as an Adjuvant

The production of chitin microparticles consisted of sonicating pure chitin powder (C-7170 Sigma) dispersed in distilled water followed by filtration through a 40μm sieve (BD Falcon Mexico) and subsequent centrifugation at

2800×g for 10 minutes. A centrifugation step at 2800×g during 10 minutes collected the microparticles which had sizes under 40 µm. A solution with 100 µg/100 µL concentration of chitin microparticles was achieved through their resuspension in distilled water after drying at 40°C. The preparation of autoclaved chitin suspension occurred at 4°C for storage prior to its use. Particle size determination and distribution evaluation occurred through a laser particle size analyzer from Malvern Master Sizer based in the UK (de Figueiredo *et al*., 2015). The Limulus Amebocyte Lysate (LAL) kit from Cambrex tested both sterility and endotoxin absence in the prepared material. The absence of coagulation in the Limulus Amebocyte Lysate test results confirmed the endotoxin-free nature of the chitin-ABOR mixture (Starr *et al*., 2008). The entire adjuvant preparation process as well as testing operations ran within research laboratories based in Iraq.

Immunization Protocol

The registered biomedical research center in Iraq provided Guinea pigs (Cavia porcellus) that received maintenance under controlled environmental conditions with temperature set at 22 ± 2°C and a humidity level between 50 ± 5% under a 12 hour light/dark cycle schedule. The five-week-old animals with 300 ± 20 g weight received unrestricted food and water supply. The study included three animals per experimental group which received subcutaneous vaccine injections at ten-day intervals through 200 µL doses of vaccine formulations. The study utilized five different groups which received administration of either ABOR polypeptide with chitin adjuvant, commercial RB51 vaccine or chitin alone or ABOR polypeptide alone while serving as the negative control the animals received phosphate-buffered saline (PBS). Chitin-adjuvanted ABOR polypeptide and RB51 commercial vaccine along with their control treatments received a third subcutaneous immunization before spleen sample retrieval (Hess *et al*., 1998).

Lymphocyte Isolation and Culture

End of the immunization protocol required cardiac puncture blood collection while animals received anesthesia. The extraction process of spleens started with sterility protocols for preparing splenocyte isolation through Ficoll 1074 (Sigma) density gradient centrifugation. Separated lymphocytes received RPMI-1640 Biosera medium to which the penicillin solution contained 100 U/mL whereas streptomycin solution reached 100 µg/mL. The lab cells received incubation at 37°C inside a humidity chamber with 5% CO_2 atmospheric concentration and 80% humidity rate control. The researchers performed all immunological experiments in three replicate conditions to achieve high accuracy and repeatability (Peña-Blanco and García-Sáez, 2018). The Iraqi institutional guidelines authorized both the experimental procedures and the methods for animal management.

Assessment for ABOR-Specific IgG Levels via ELISA

Specific IgG levels within serum for vaccinated animals were ascertained through means for an enzyme-linked immunosorbent test (ELISA). within PBS, 96-well plates were briefly covered alongside various doses for the pure ABOR protein, and overnight incubated for 4°C. Three times the plates were washed; then, for room temperature, they were blocked for one hour using blocking buffer. Each well received 100 µL for diluted serum samples (1;500, and 1;200) subsequent to extra washing, and incubated for room temperature for 2.5 hours. The wells were cleaned, furthermore horseradish peroxidase (HRP)-conjugated anti-guinea pig IgG (1;10000 dilution within PBS) added, and incubated for one hour. Three, three, five, five'-Tetramethylbenzidine (TMB) substrate (Sigma) used to be used for colorimetric detection; the reaction used to be halted alongside 50 µL for 2N H_2SO_4. Measuring optical density (OD) for 450 nm alongside a reference wavelength for 630 nm, Du *et al*. (2000) found.

Cytokine Gene Expression Analysis Using RT-qPCR

Following manufacturer directions, total RNA used to be isolated coming from blood samples kept within RNAlater solution using a Gene All Hybrid-R RNA Purification Kit (South Korea). to remove genomic DNA contamination, the RNA used to be processed alongside 5 U for amplification-grade DNase I (Fermentas, Lithuania.). A NanoDrop-1000 spectrophotometer (Thermo Scientific) (Vemulapalli *et al*., 2000) evaluated purity, and concentration. M-MuLV reverse transcriptase (Fermentas, Lithuania) used to be used for cDNA synthesis; SYBR Green I mix (Roche Applied Sciences, Germany) used to be used for quantitative PCR within a StepOneTM instrument (Applied Biosystems). Denaturation for 95°C for 15 s, annealing for 60°C for 10 s, and extension for 72°C for 15 s for 45 cycles comprised the amplification settings. Using GAPDH as a housekeeping gene, gene expression used to be normalised; relative fold expression used to be furthermore analysed using the 2^−ΔΔCt approach (Takebe *et al*., 2003). The primers used within the RT-qPCR analysis are shown within the following Table 1.

This table emphasizes the particular primers used for amplifying genes linked to cytokine generation, which are essential for immunological regulation during a brucella infection as shown within the following Table 2. Designed using publicly accessible GenBank databases, the primer sequences were tested for efficiency, and specificity (Obrani *et al*., 2013).

Lymphocyte Proliferation Assay via Flow Cytometry

Carboxyfluorescein succinimidyl ester (CFSE) staining let

one assess splenocyte proliferation. Labelled alongside 5 µM CFSE (Molecular Probes, USA), spleenocytes were incubated for 37°C, 5% CO_2, furthermore examined beneath a flow cytometer 72 hours later. Comparing the fluorescence intensity for experimental, and control groups allowed one to ascertain the proliferation index (PI) (Kim *et al.*, 2016).

Table 1: The primers used within cytokine genes expression level assay through Real-Time RT-PCR.

Gene	Forward (5′-3′)	Reverse (5′-3′)
IL-4	tgacggtcattctcttctgcctc	aggagagtgtgtgttgaggtgctg
IL-12	acctccctagggcctcaccag	ggccttgtgaagttcactgttc
IL-5	ggaagctctggcaacactattc	tgcttcactctccgtcgctcc
IL-10	gccacccagagcagaccac	accctgccaaaggcagctcgg
TNF-α	tgcactttggggtgatcggcc	agccaccggcttgtcattatcg
IFN-γ	atgttggctcttcagttctg	catctgagttatctgcattc
IL-2	gttatgctttctcagagcaaccc	gctaaatttagcacctgctccac
GAPDH	gtagccatcaatgatccct	aaggctgagaatgggaagct

Statistical Analysis

Every experiment used to be run within triplicate; results were reported as mean ± standard deviation (SD). One-way analysis for variance (ANOVA) alongside Tukey's post-hoc test used to be used to find statistical significance; p-values <0.05 were regarded statistically significant (Seleem *et al.*, 2004). REST 2009 program (Qiagen, Germany) (Glomski *et al.*, 2002) used to be used to examine real-time qPCR data.

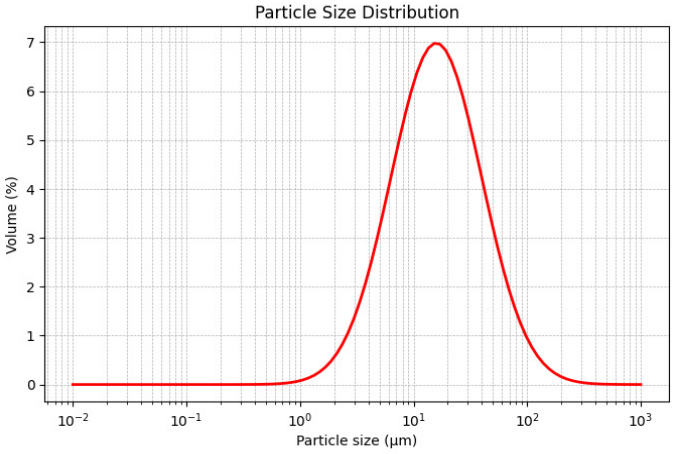

Figure 1: Particle size distribution for chitin microparticles.

RESULTS

Particle Size Distribution for Chitin Microparticles

The particle size distribution for chitin used to be investigated using a laser particle size analyzer within order to assess its adjuvant usefulness. The findings showed, that 90% for the chitin microparticles were less than 64.5 µm, 50% were below 23.6 µm, and 10% were lower than 6.01 µm, therefore suggesting an ideal size range for phagocytosis, and immunological activation. Figure 1 shows the size distribution profile, alongside a peak between 10–20 µm, therefore verifying the homogeneity for the microparticles.

Table 2: Summary for key findings.

Parameter	PBS Control	Chitin Alone	RB51 Vaccine	ABOR Alone	ABOR +Chitin
IgG Absorbance (450 nm)	Low	Moderate	High	Moderate	Highest
Cytokine Expression (IFN-γ, IL-12, TNF-α)	Low	Moderate	High	Moderate	Highest
Lymphocyte Proliferation (PI)	Low	Moderate	High	Moderate	Highest
IFN-γ/IL-10 Ratio	Low	Moderate	High	High	Highest
Histopathology (Liver Toxicity)	No damage	No damage	No damage	No damage	No damage

Humoral Immune Response; IgG Absorbance Levels

Guinea pigs immunised alongside ABOR polypeptide, and RB51 were used within the ELISA experiment to evaluate IgG-specific antibody levels. The results—shown within Figure 2—show, that compared to the PBS control group, ABOR + chitin, and RB51 groups had substantially greater IgG absorbance values. Particularly, the mean absorbance for 450 nm used to be 2.051 ± 0.217 for ABOR + chitin, and 1.864 ± 0.115 for RB51, proving that ABOR polypeptide may produce an equivalent antibody response to the commercial RB51 vaccination.

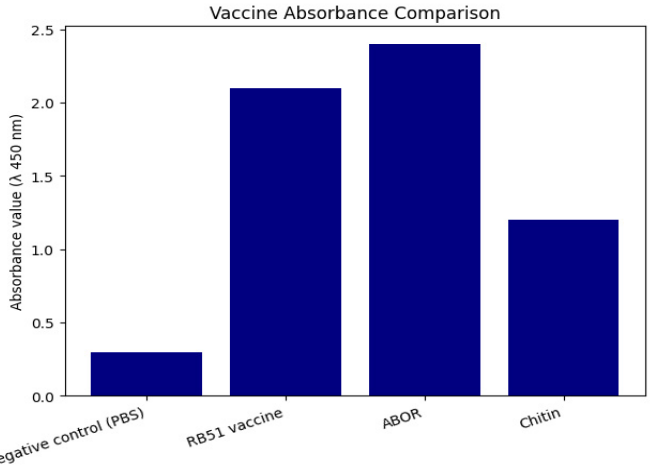

Figure 2: Vaccine absorbance comparison.

Serum Absorbance Levels within Infected and Non-Infected Cattle

Sera coming from healthy, and Brucella-infected calves were examined for IgG binding upon ABOR-coated plates to verify the immunogenicity for ABOR polypeptide within a genuine infection environment. Sera coming from infected calves had much higher absorbance values (mean 0.601 ± 0.218) than those coming from healthy animals (mean 0.218 ± 0.078, p = 0.0001, Figure 3).

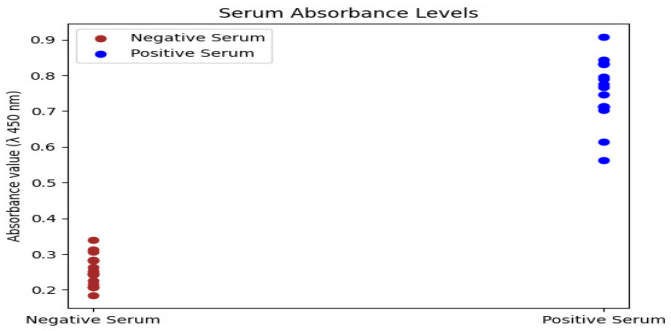

Figure 3: Serum absorbance levels within infected and healthy cattle.

Cytokine Gene Expression and Th1/Th2 Polarization

Real-time RT-PCR was used to evaluate the expression for important cytokines including IFN-γ, IL-2, IL-12, TNF-α (Th1 cytokines), and IL-4, IL-5, IL-10 (Th2 cytokines), thereby assessing the immune response for the molecular level. Figure 4 shows ($p < 0.05$), that within the ABOR + chitin group, IFN-γ, IL-12, and TNF-α were considerably raised compared to the RB51 group. Essential for Brucella clearance as well as cell-mediated immunity, these cytokines are also found within upon the other hand, the ABOR + chitin group considerably downregulated IL-10, IL-5, and IL-4 (which are linked alongside Th2 responses), hence showing a Th1-skewed immune response.

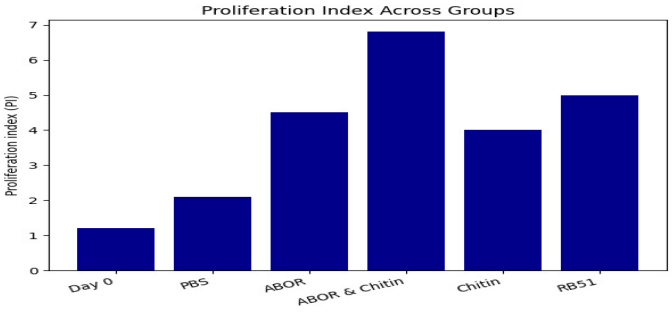

Figure 4: Gene expression levels for key cytokines.

Lymphocyte Proliferation Index

Using a lymphocyte proliferation test, we assessed cellular immune responses. The proliferation index (PI) for splenocytes within many groups is shown within Figure 5. The lowest proliferation rate used to be coming from the PBS control group; ABOR + chitin produced the greatest PI (≈6.8), followed through RB51 (≈5.2).

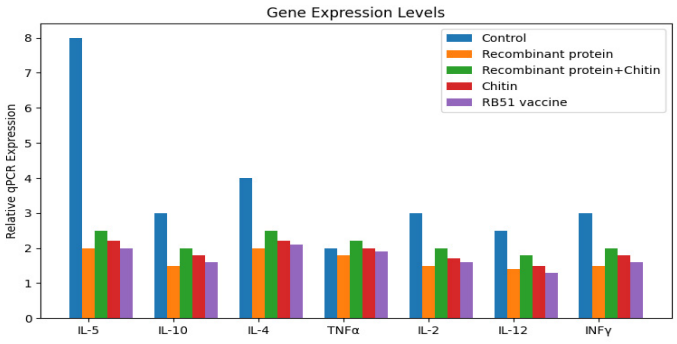

Figure 5: Proliferation index across groups.

IFN-γ/IL-10, and IFN-γ/IL-4 Ratios; Confirmation for Th1 Response

Calculated ratios for IFN-γ to IL-10, and IFN-γ to IL-4 might help to substantiate the Th1-skewing impact for the vaccination candidates even further. These ratios were significantly greater within the ABOR + chitin group than within RB51, and control groups, Figure 6 reveals.

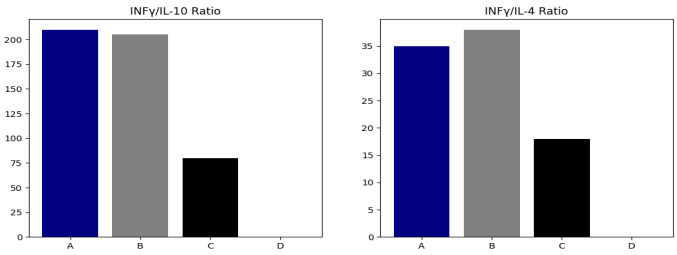

Figure 6: IFN-γ/IL-10, and IFN-γ/IL-4 ratios.

Histopathological Evaluation for Liver Tissues

To evaluate possible toxicity for the ABOR polypeptide, liver tissues were histologically examined. Across experimental groups, no notable necrotic alterations, degeneration, or hepatocellular injury used to be seen.

DISCUSSION

Particle Size Distribution Analysis

These findings are in tandem with previous findings on effective activation of the antigen-presenting cells aided by smaller chitin microparticles (Lee *et al.*, 2002). The percentage of the particle size analysis of chitin microparticles revealed that ninety percent of all particles were 64.5 1/16 less, fifty percent was under 23.6 1/16 and ten percent was under 6.01 1/16. These findings indicate that in the phagocytosis process via an antigen presenting cell (APCs), the majority of the chitin particles fall within an optimal size range- which is instrumental in effective activation of the immune system (Lee *et al.*, 2002). Via macrophage, and

dendritic cell activation, chitin, with its reported immunomodulating properties, has been proven to enhance innate, and adaptive immune response, as such promoting the expulsion of pro-inflammatory cytokines such as TNF- IL-12 (Puspitoyani *et al*., 2020). The extremely small size of these microparticles ensures that microparticles are efficiently absorbed via APCs which facilitates sustained display of antigen, and triggers Th1 responses a characteristic that is imperative in combating intracellular diseases such as Brucella abortus (Schurig *et al*., 2002). And the optimum particle size pretends that the microparticles of chitin can be effectively used as adjuvant in vaccination preparations, thus enhancing antigen containment, and augmenting the persistence and duration of the immune response.

Humoral Immune Response Implications

This implies, that a significant humoral immune response (Vemulapalli *et al*., 2000) may be generated through the recombinant antigen within concert alongside chitin. The ELISA findings showed, that the ABOR protein, especially within conjunction alongside chitin, produced a robust humoral immune response alongside absorbance values far above the PBS control, and similar to the RB51 vaccination. Indicating the great immunogenicity for the recombinant multi-epitope vaccination, the mean absorbance for 450 nm for the ABOR+chitin group (2.051 ± 0.217) used to be rather greater than, that for the RB51 vaccination (1.864 ± 0.115). This implies, that ABOR is able to induce the synthesis for antigen-specific IgG, a fundamental component for protective immunity against Brucella infection (Vemulapalli *et al*., 2000). through improving antigen presentation, and extending immune activation, chitin's usage as an adjuvant most certainly helped to produce this response. Effective Brucella vaccines must generate strong, and lasting antibody-mediated immunity, hence the ABOR+chitin formulation shows a good choice for further research (Dorneles *et al*., 2015). Investigating its capacity to provide long-term defense against Brucella infection within cattle should be the main emphasis for further investigations.

Natural Infection Response Validation

This signifies, there are epitopes that can be recognized by the immune system spontaneously during an infection, thus it confirms that ABOR can be used as a possible vaccination antigen (Datta *et al*., 1990). The serological analysis of the infected, and healthy cattle serum indicated significantly high absorbance levels of IgG in the infected demographics (0.601 + 0.218) comparatively to the healthy controls (0.218 + 0.078) with $p < 0.0001$. This indicates, that the ABOR protein contains immunodominant epitopes, naturally identified by the immune system in an infection, thus indicating potential application as a diagnostic antigen or a vaccine component (Datta *et al*., 1990). in the context, that as a subunit vaccine, the capability of the ABOR protein to elicit potent antibody response in naturally occurring animals implies potentially high efficacy protection. Moreover, highlighted by these findings is the imperativeness of the selection of antigenic targets, which would be similar to the actual infection in order to ensure robust, and long-term protective immunity. in addition to the establishment of the protective efficacy of ABOR, future studies should also explore the functional properties of these antibodies, including antigen in opsonisation and bacterial clearance (Monreal *et al*., 2004).

Cytokine Profile and Immune Polarization

In a Brucella vaccination, such polarization is optimal since Th1 mediated responses correspond with protective immunity (Zhu *et al*., 2011). Analysis of ABOR + chitin treatment produced a greater output in Th1 cytokines (IFN-y, IL-12 and TNF-y) above those of RB51 and control groups as real-time RT-PCR results indicated. The IL-12 proteins and high IFN-gamma also stimulate the antigen-specific trigger action of cytotoxic T cells and macrophages (Zhu *et al*., 2011). The IL-10, IL-5 and IL-4 levels were lower in mice exposed to ABOR+chitin implying a diminished Th2 response activity that is commonly found in chronic infections of Brucella (Khorramizadeh *et al*., 2019). Optimum immune control over Th1 reactions must be obtained through the development of clinical vaccines that are guided by the previous needs regarding anti-Brucella defenses (Jacob and Curtiss, 2021). The protection ABOR+chitin combination against RB51 provokes is superior since it increases cell-mediated immunity, which is necessary to eliminate intracellular bacteria.

Cellular Immune Response Assessment

This would suggest, that ABOR polypeptide, particularly, when combined with chitin, efficiently primes the expansion of T-cells, the essential attribute of a robust adaptive immunological response (Rieger *et al*., 2011). Analysis of the lymphocyte proliferation assay indicated that the splenocytes taken from the ABOR(+)chitin inoculated guinea pigs had the proliferation index of ~6.8 and was significantly higher compared to that of the RB51 vaccinated guinea pig that had the proliferation index of ~5.2. The desired cellular defense to this antigen-specific response is evidence that the administration of ABOR formulation is a product that elicits protection against intracellular infections such as Brucella (Rieger *et al*., 2011). The immunostimulatory characteristics of chitin allow the APCs to take up antigens and present them to the T-helpers cells resulting in the increased T-cell activation as per Ugalde *et al*. (2003). ABOR-chitin combination signifies the possible utility as a superior vaccine because cellular immunity is essential to have in control of Brucella infections and the cellular immune response this vaccine construct produces is, in fact, more than that produced by RB51 Vaccine.

Th1 Response Confirmation and Clinical Implications

The IL-4-mediated Th2 responses are decreased by IFN- g as a result of which this fact establishes, the predominant induction of Th1 immune response by ABOR polypeptide- is the key event in the effective clearance of Brucella (Ugalde *et al*., 2003). As expected, a strong Th1-polarized immune system was corroborated by low IFN-/IL-10, and IFN-/IL-4 ratio in the AAOR+chitin incubated group, as compared to the RB51, and control groups. Although IL-10, and IL-4 are associated with immunosuppressive, and Th2 respectively, IFN- (interferon gamma) is an essential cytokine in intracellular killing of bacteria (Babaoglu *et al*., 2018). The more elevated IFN-gamma/IL-10, and IFN-gamma/IL-4 ratio also indicates, that the ABOR vaccination design promotes protective immunity, thus reducing the risk of persistent infection (Schurig *et al*., 2002). By reducing IL-10 immunoregulatory effect that is found to be high in chronic Brucella infection, ABOR+-chitin then may provide increased protection as compared to RB51. A higher carrying out of large-scale animal experiments should be done to evaluate the long-term effects of this immunological polarization to bacterial clearance, and prevention of illnesses.

Safety Profile and Overall Assessment

This confirms the safety designation of ABOR polypeptide as a vaccination agent because it means, that obvious toxicity cannot be attributed to it (de Souza Filho *et al*., 2015). Results of this study highlight the immunogenic potential of the ABOR polypeptide as an alternative to the RB51 vaccination. The ABOR + chitin combination elicited high levels of IgG antibodies; vigorous T-cell stimulation and a Th1-predominant cytokine response to Brucella abortus; each of which is a fundamental requirement in the development of effective protection against Brucella abortus. Absence of toxicity adds to its more viability as effective and safe vaccination alternative. Further studies ought to be focused on the achievement of an evaluation of protective efficacy through the use of challenge tests on cattle models.

CONCLUSIONS AND RECOMMENDATIONS

Strong, and protective immune response against Brucella abortus is produced through the recombinant multi-epitope polypeptide vaccination (ABOR) within conjunction alongside chitin microparticles, the research showed. Essential for intracellular pathogen clearance, ABOR+chitin produced more IgG levels, improved cytokine production promoting Th1-mediated response, and more lymphocyte proliferation than the RB51 vaccination. Significantly, histological analysis verified the lack for toxicity, therefore supporting its possible use as a safe vaccination candidate. More research should concentrate upon assessing ABOR's long-term protective effectiveness within big animal models, including cattle, beneath field circumstances, thus advancing the development for ABOR as a feasible brucellosis vaccination. Furthermore, improving immunogenicity might be adjusting the adjuvant composition and evaluating many administration methods. Given the relevance for cellular immunity within brucellosis control, further studies should investigate the capacity for the vaccination to produce long-lasting memory responses. Moreover, combining proteomic, and genomic techniques can enable better choice for epitope for next-generation subunit vaccines. In the end, this work addresses safety issues, and shows the potential for ABOR+chitin as a viable substitute for live-attenuated vaccines, hence generating significant immune responses. Its further evolution, and confirmation might greatly help to manage brucellosis within areas for endemicity.

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NOVELTY STATEMENT

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AUTHOR'S CONTRIBUTIONS

Merriam Ghadhanfar Alwan: Design of study. Wameedh Hashim Abbas Alqatrani: ELISA and microbiology. Protein work. Ibrahim Ayad Jihad. Qais R. Lahhob: Animal experiments. Muhib: RT-qPCR. Hasan Ali Alsailawi: Head of the project and article. Mustafa Adnan Zaidan: Flow cytometry. Every author gave consent to the manuscript.

Conflict of Interest

There is no conflict of interest by the authors.

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