Original Article

Intranasal versus intradermal immunoprotective effect of maltodextrin nanoparticles loaded with SAG1 against toxoplasmosis in murine model

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ABSTRACT

Background: Until now, there is no available efficient vaccine against toxoplasmosis, and the current medications have adverse side effects. Therefore, the search for prophylactic strategies against toxoplasmosis is mandatory.
Objective: To evaluate the immunoprotection potential activity of the immunogenic *T. gondii* surface antigen 1 (SAG1) combined with maltodextrin nanoparticles (MNPs) against toxoplasmosis. A secondary objective is to assess the most efficient route of administration, intranasal (IN) or intradermal (ID).
Material and Methods: This study was carried out on 50 Swiss albino mice that were equally divided into 5 groups: negative and positive controls, ID immunized with SAG1, IN immunized with SAG1-loaded MNPs, and ID immunized with SAG1-loaded MNPs. Brain cyst counting, histopathological examination, and measurement of immunoglobulins G, and A, and interleukins 10, and 12 levels were used to assess the immunoprotective effects SAG1-loaded MNPs.
Results: The efficacy of immunization with SAG1 was enhanced after loading it on MNPs with significant reduction rate of brain cyst count: 89.76% in the ID immunized group, and 77.46% in the IN immunized group. Moreover, SAG1-loaded MNPs IN immunized group showed the least pathological changes and the highest levels of anti-*T. gondii* IgG with the highest levels of cytokines (IL-10 and IL-12).
Conclusion: Maltodextrin NPs are a promising effective delivery tool for SAG1. Since IN route is a needle-free method of administration, it is preferred than ID route.

Keywords: cytokines; immune response; maltodextrin; NPs; SAG1; toxoplasmosis; vaccination.

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INTRODUCTION

Toxoplasmosis is a worldwide disease that affects a wide range of warm-blooded species, including humans. Due to variable routes of infection, it is one of the most prevalent diseases of great importance in veterinary and medical fields[1]. The genetic background, immunological condition, and *T. gondii* strain are the primary factors determining host immune response. Although there is considerable knowledge concerning the biology, epidemiology, and molecular host-parasite interactions, effective control strategies to avoid infection are scarce which highlighted the need for vaccination[2]. Various vaccines were designed for long-term protection including inactivated[3], attenuated[4], subunit[5] or recombinant-designed[6], and DNA vaccines[7]. The immunogenicity of live-attenuated vaccines proved to be significantly affected by the degree of attenuation. Furthermore, the utilization of these vaccines was limited because of their insufficient shelf-life and their potential reversion to a pathogenic state. SAG1 is a specific surface membrane protein of *T. gondii* tachyzoites that is essential for parasite adhesion, host-cell penetration and has an important role in immune modulation[8].

Only a few research studies investigated how the chosen vaccination method affects its effectiveness. The method of inoculation can impact the spread of a vaccine, allowing it to activate immune cells and trigger immunological responses in both local and systemic areas[9-11]. Mucosal vaccines elicit protective antigen-specific mucosal and systemic immune responses superior to parenterally delivered vaccines. In addition to their valuable immunogenicity, mucosal vaccines are easier to administer without a need for injection and administration with highly trained personnel[12,13].

The SAG1 protein is a highly immunogenic antigen that serves the initiation of molecular host-parasite interaction. It is the most favorable option for developing a potent vaccination against toxoplasmosis due to its ability to activate both the cellular and humoral immune responses[14].

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The utilization of potent immunogenic antigens in conjunction with NPs that serve as both adjuvants and delivery vehicles, showed great potential for the advancement of a successful vaccine in the future[15]. Furthermore, NPs not only protect against degradation, but also allow for targeted delivery to specific cells by attaching antibodies to their surfaces. This allows for an efficient transportation system *via* different paths besides subcutaneous or intramuscular delivery[16,17]. Besides, NPs can also be engineered for non-invasive delivery, such as IN immunization, which has emerged as a reliable alternative to the injection-based route of administration. In fact, NPs delivering vaccines offered a more convenient tool for large campaigns of vaccination[18].

Cationic reticulated MNPs possess a phospholipid core and have a porous spherical structure. They carry a significant amount of antigen and distribute it to both epithelial and immune cells. Because of their anionic lipid core, MNPs can permeate the mucus when administered nasally, resulting in an increased duration of the antigen existence in the nasal mucosa. Consequently, they are reportedly well-suited for nasal immunization[19,20]. Therefore, we hypothesized that loading SAG1 with MNPs could elicit effective immunoprotective effects against toxoplasmosis in experimental mice. We also assessed the IN and ID routes of administration.

MATERIAL AND METHODS

This experimental case-control study was conducted at Theodor Bilharz Research Institute (TBRI) in Giza, Egypt during the period from February to May 2022.

Study design: The ME-49 strain was maintained in our laboratory to prepare *T. gondii* SAG1 that was loaded on MNPs. Mice were immunized with three doses of the prepared vaccine by the ID or the IN route at two weeks intervals. Immunized mice were infected with *T. gondii* ME-49 strain, and sacrificed after two months. Parameters used for evaluating vaccine efficacy include brain cyst count, histopathological examination, and immunological assessment.

Experimental animals: The study utilized 50 Swiss-albino laboratory-bred female mice aged 6–8 weeks and weighing an average weight of ~20-25 grams obtained from TBRI. The mice were maintained within the animal facility at TBRI in a controlled environment that was free from pathogens and following standard housing protocols.

Study groups: Mice were divided into five equal groups; normal control group (GI), positive control group (GII), ID immunized group with SAG1 alone (GIII), IN immunized group with SAG1-loaded MNPs (GIV) and ID immunized group with SAG1-loaded MNPs (GV).

Preparation of SAG1[21]: *T. gondii* RH strain tachyzoites were obtained from the Parasitology Department at TBRI, and maintained in three male Swiss-albino mice by intraperitoneal passage every four days. Peritoneal fluids from the infected mice were collected in saline and passed through a 3 um nucleopore filter to remove host cells. The filtered fluid was pooled and centrifuged at 2000 rpm for 20 min, washed three times with phosphate buffer saline (PBS), and disrupted for one hour on ice by an ultrasonic disintegrator. The disintegrated fluid was observed by light microscopy to ensure absence of whole parasites and centrifuged at 12000 rpm for one hour. The supernatant was collected as soluble antigen, and SAG1 was purified using anion-exchange liquid chromatography[21].

Preparation of MNPs[22]: Maltodextrin (dextrose equivalent, DE 10) was dissolved as maltodextrin Milli-Q water phase in emulsifier (SDS, Tween 80, and Span 80, respectively), obtained from Sigma Chemical Company USA). It was added to the suspension with a syringe pump (Legato, Kd scientific, US) whilst stirring. A fixed quantity 80, 100, and 120 ml of absolute ethanol, was added dropwise to 10 ml of the above suspension and continually stirred using a magnetic stirrer. Samples of the nanoparticles were obtained by centrifugation, rinsed with absolute ethanol three times to remove excess water and emulsifier, and then 0.5%, w/w of the three polysaccharides: tea polysaccharide (TP), pumpkin polysaccharide (PP), and balsam pear polysaccharide (BP), were dissolved in the maltodextrin solution. The MNPs loaded with TP, PP, and BP were called MNPs-TP, MNPs-PP, and MNPs-BP[22].

Preparation of SAG1-loaded MNPs[23]: Purified SAG1 was mixed with MNPs at a 1:3 weight ratio (*e.g.*, 15 µg SAG1 with 45 µg MNPs). The SAG1-loaded MNPs were dissolved in water at room temperature, 20 µl for the IN route and 50 µl for ID[23].

Immunization schedule: Mice were vaccinated with three doses of SAG1-loaded MNPs by the ID route (50 µl) or the IN route (20 µL) at two weeks intervals[23].

Challenge by infection and mice scarification: All mice except the negative control group were infected by intraperitoneal injection with 0.1 ml of brain suspension containing $1x10^2$ cysts per ml of previously infected mice with *T. gondii* ME-49 strain. The number of cysts were counted by hemocytometer under a light microscope. Non-infected negative control groups were instead inoculated with PBS. The mortality rate was recorded throughout the study period. Two months after the infection, peripheral blood samples were aseptically collected for further study tests and all survived mice were euthanized with inhaled

isoflurane and sacrificed by cervical dislocation and then dissected to obtain brain, muscle and eye. The collected tissues were preserved in 10% neutral buffered formalin solution for histological analysis[24].

Detection of *T. gondii* in the brain: The brain tissue was divided into two parts. One part was blended with one ml of a 10% formalin solution. Intact cysts in a 100 µl specimen of each homogenate were counted using a light microscope. The mean number of cysts in each group was calculated using the formula: cyst count in 100 µl ×10×2[25]. The reduction rate of brain cyst count was measured using the formula: (mean value of brain cyst count of the infected untreated group - mean value of brain cyst count of the infected treated group) X 100/mean value of the infected untreated group[24].

Histopathological assessment: Specimens of the brain, heart, and eye were dehydrated in different concentrations of alcohol, cleared with xylol, and then embedded in paraffin blocks. Four-micron thick sections of paraffin-embedded samples were stained with hematoxylin and eosin (H&E)[26].

Measurement of serum anti-*T. gondii* IgG and IgA using ELISA: Anti-mouse toxoplasmosis antibodies IgG ELISA Kit (Cat. No: NBP2-60169 Novus Biologicals, USA) and IgA (TP-IgA) kit (CAT. No: MBS1610482 MyBioSource, USA) were used to detect IgG and IgA respectively in sera of mice as per the manufacturer's instructions. Diluted sera were added to the *T. gondii* antigen-coated microtiter plate and then specific anti-mouse antibodies conjugate were added. After incubation and repeated washing, the chromogenic substrate was added[27]. The optical densities were read at 450 nm by an ELISA reader (MR-96A, Mindray Bio-Medical Electronics, China)

Measurement of cytokines: Both IL-10 and IL-12 were measured using ELISA. The IL-10 mouse ELISA kit (Cat. No: ab100697. Abcam, Cambridge.UK) and mouse IL-12 p40 Simple Step ELISA® Kit (Cat. No: ab236717. Abcam, Cambridge.UK) were used, respectively as per the manufacturer's instructions. Dilution of test samples, negative control, positive control, and calibrators were added to each of IL-10 and IL-12 coated wells, followed by incubation and repeated washing. Enzyme conjugate (100 µl) was dispensed to each well and mixed gently for 10 sec then incubated at 37○C for 30 min and washed. The TMB One-Step development solution and then stop solution were added to each well[28]. Optical density was read at 450 nm immediately by ELISA reader (MR-96A, Mindray Bio-Medical Electronics, and China).

Statistical analysis: Data were encoded and analysed using the Statistical Package for Social Sciences (SPSS), version 22.0 (Armonk, NY: IBM Corp.). Kruskal-Wallis's test was used to determine if there were statistically significant differences between the groups and Post hoc test was used as a multiple comparison test to assess inter-group differences. Values were considered statistically significant when $P<0.05$.

Ethical considerations: All animal studies were performed according to the guidelines for animal experimentation (EU Directive 2010/63/EU), and after being approved by the ethical committee of the National Liver Institute (NLI IRB 00003413 FWA0000227).

RESULTS

Mortality rate: The mortality rate was 10% in the negative control group, 20% in the positive control group, 10% in SAG1 ID immunized and SAG1-loaded MNPs IN immunized groups, whereas the highest mortality rate of 30% was in SAG1-loaded MNPs ID vaccinated group (Fig. 1).

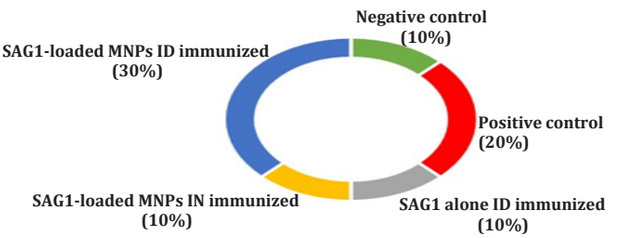

Fig. 1. Mortality rate in the studied groups.

The reduction rate of brain cyst count: The best reduction efficacy regarding brain cyst count was recorded in SAG1-loaded MNPs immunized groups, with a reduction rate of 89.76% in the ID immunized group (GV) and 77.46% in the IN immunized group (GIV) and the lowest reduction rate of 62.43% was present in SAG1 alone ID immunized (GIII) (Table 1, and Fig. 2).

Histopathological evaluation

- **Brain:** Brain sections of positive control group showed severe meningeal inflammation, the presence of granuloma, moderate vasculitis, and moderate gliosis. SAG1 alone immunized mice showed moderate meningeal inflammation, severe vasculitis, severe neurodegenerative changes, and mild gliosis. SAG1-loaded MNPs ID immunized mice showed moderate meningeal inflammation, mild vasculitis, absence of granuloma, absence of edema, moderate gliosis, and severe neurodegenerative changes. Brain sections of SAG1-loaded MNPs IN immunized mice showed the least pathological changes: mild meningeal inflammation, mild gliosis, absence of granuloma, and absence of edema (Fig. 3 A-D).

- **Heart muscle:** The positive control group showed mild neurodegenerative changes, and severe inflammation, while the SAG1 alone immunized group and SAG1-loaded MNPs IN immunized group showed the least pathological changes: absence of

Table 1. Brain cysts count and reduction percentage between the studied groups.

	Number of cysts Mean ± SD	Reduction rate %
Groups		
Negative control (GI)	0.00 ± 0.00	--
Positive control (GII)	257.33 ± 44.7	--
SAG1 alone ID immunized (GIII)	96.67 ± 81.43	62.43%
SAG1-loaded MNPs IN immunized (GIV)	58.0 ± 42.29	77.46%
SAG1-loaded MNPs ID immunized (GV)	26.33 ± 2.87	89.76%
Statistical analysis		
Kruskal Wallis test	14	
***P* value**	0.0001*	

*: Significant ($P<0.05$).

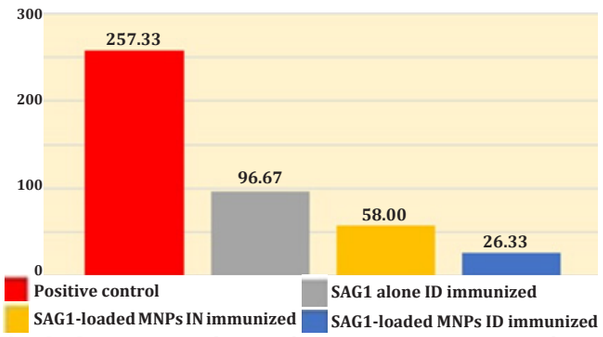

Fig. 2. Comparison of mean brain cyst count among the studied groups.

tissue cysts, mild neurodegenerative changes, and mild inflammation. Tissue cysts were absent in the SAG1-loaded MNPs ID immunized group which however showed severe neurodegenerative changes, and moderate inflammation (Fig. 4 A, B, C, D).

- **Eye:** Histopathological examination of the eye of positive control group showed mild necrosis, and severe inflammation. SAG1 alone immunized group showed absence of tissue cysts, mild necrosis, and mild inflammation. Regarding SAG1-loaded MNPs ID immunized mice showed the absence of tissue cysts, severe necrosis, and moderate inflammation. SAG1-loaded MNPs IN immunized mice showed the least pathological changes; absence of tissue cysts, mild necrosis, and mild inflammation (Fig. 5 A, B, C, D).

Immunological assays

- **Humoral immune response (IgG and IgA levels):** The highest level of IgG among the three immunized groups was reported in the SAG1-loaded MNPs IN immunized group (1.16 IU/ml) followed by the SAG1-loaded MNPs ID immunized group (1.09 IU/ml) and the least level of IgG (0.33 IU/ml) was recorded in the SAG1 ID immunized group. Regarding the level of IgA, the highest level (1.62 IU/ml) was in the SAG1-loaded MNPs ID immunized group followed by SAG1-loaded MNPs IN immunized group (1.49 IU/ml) and the least level (0.04 IU/ml) in SAG1 ID immunized group (Fig. 6 A, B).

- **Cytokines assays (IL-10 and IL-12):** Among the immunized groups, the highest level of IL-10 (47.4

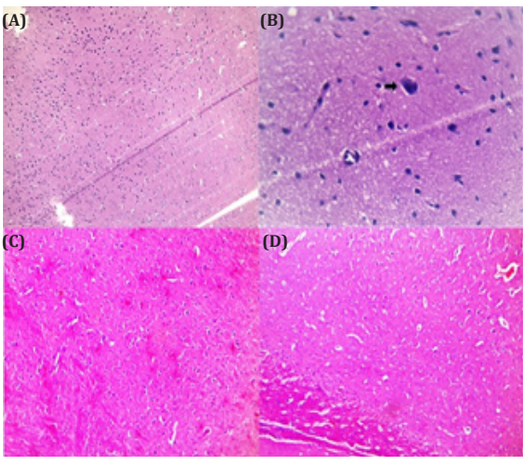

Fig. 3. Cross sections of brain from the study groups. **(A)** Negative control group showed normal brain tissue, normal neurons, and no inflammation. **(B)** Positive control group showed cyst of *T. gondii* (black arrow), strong inflammatory reaction, parenchymal mononuclear infiltrates, degenerative changes, and strong perivascular inflammatory cells. **(C)** SAG1-loaded MNPs IN immunized sections showed mild meningeal inflammation, mild gliosis, absence of granuloma and absence of edema **(D)** SAG1-loaded MNPs ID immunized sections showed moderate meningeal inflammation and mild vasculitis (H&E x400).

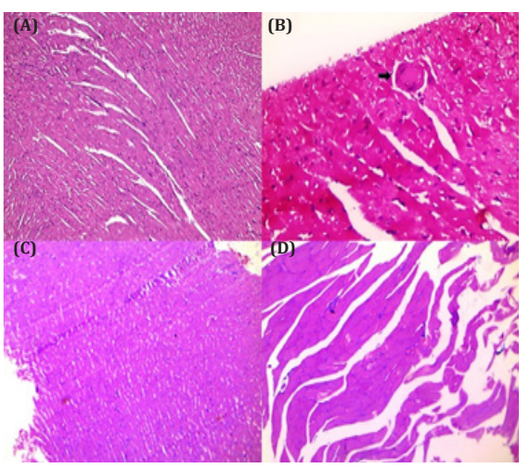

Fig. 4. Cross sections of heart muscle from the studied groups. **(A)** Negative control group showed normal muscle tissues, no cysts, and no inflammation. **(B)** Positive control group showed cyst of *T. gondii* (black arrow). **(C)** SAG1-loaded MNPs IN immunized showed mild neurodegenerative changes, and mild inflammation. **(D)** SAG1-loaded MNPs ID immunized showed mild inflammatory reaction and mild neurodegenerative changes (H&E stain x200).

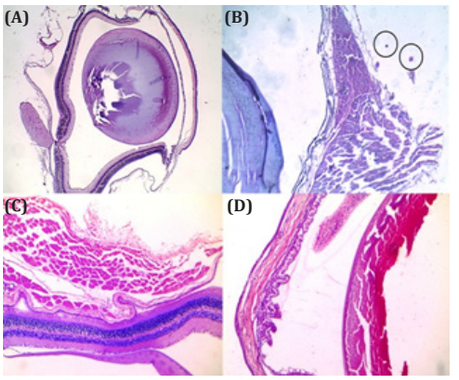

Fig. 5. Cross sections of eye from the studied groups. **(A)** Negative control group showed no pathological changes. **(B)** Positive control group showed cyst of *T. gondii* (black circles), mild necrosis, and severe inflammation. **(C)** SAG1-loaded MNPs IN immunized sections showed absence of tissue cysts, mild necrosis, and mild inflammation. **(D)** SAG1-loaded MNPs ID immunized sections showed absence of tissue cysts and moderate inflammation (H& E stain x200).

pg/ml) was in the SAG1-loaded MNPs IN immunized group followed by the SAG1-loaded MNPs ID immunized group (36.1 pg/mL); and the lowest level (26.9 pg/ml) was in the SAG1 ID immunized group. Concerning the level of IL-12, the highest level (19.7 pg/ml) was in SAG1-loaded MNPs IN immunized group followed by SAG1-loaded MNPs ID immunized group (14.2 pg/ml) and least level of IgG (12.1 pg/ml) in SAG1 ID immunized group (Fig. 6 C, D).

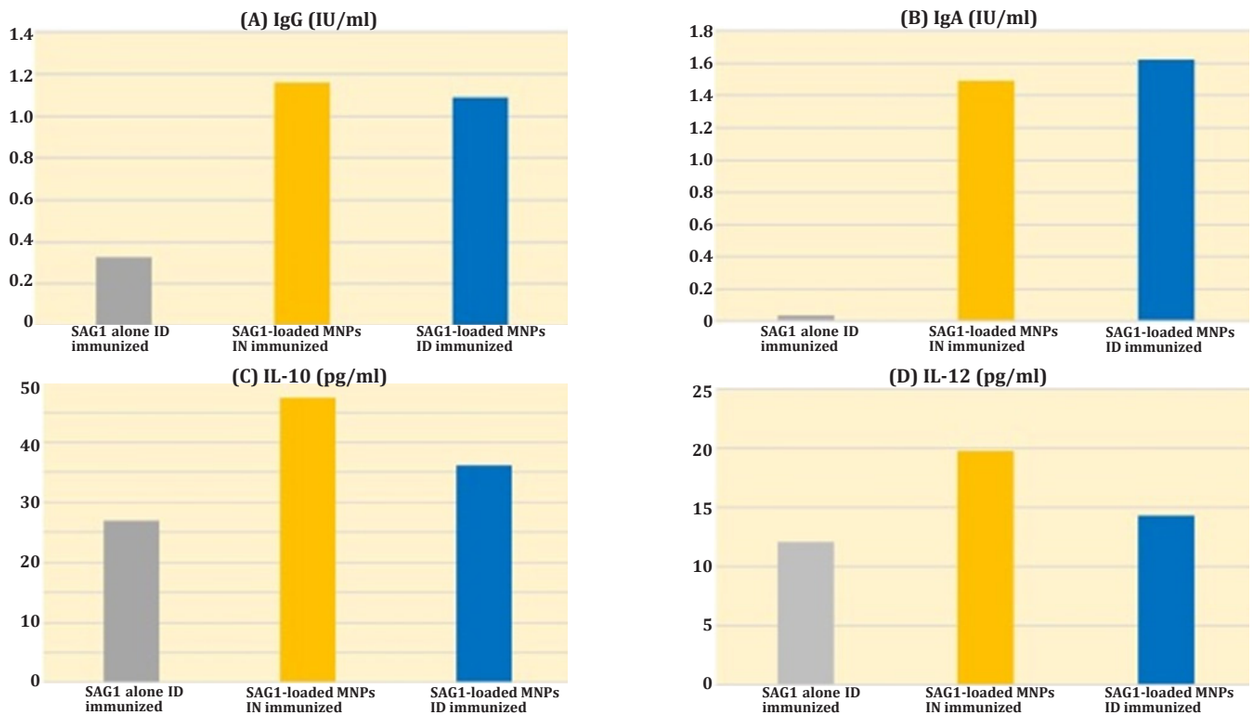

Fig. 6. (A) Mean levels of IgG by ELISA test among the immunized groups. **(B)** Mean levels of IgA by ELISA test among the immunized groups. **(C)** Mean levels of IL-10 by ELISA test among the immunized groups. **(D)** Mean levels of IL-12 by ELISA test among the immunized groups.

DISCUSSION

Lately, new approaches have been considered for the prevention of toxoplasmosis. With the rapid development of nanotechnology in biomedicine, nanoparticles have presented themselves as strong candidates for the prevention of infectious diseases, such as COVID-19, hepatitis B, and toxoplasmosis[15]. Therefore, we evaluated the efficacy of IN and ID administration of SAG1-loaded MNPs on toxoplasmosis in experimental mice. In the present study, the efficacy of immunization with SAG1 was enhanced after loading on MNPs with significant reduction of brain cyst count in the SAG1-loaded MNPs ID and IN immunized groups. These results agreed with Ducournau *et al.*[10] who found no cysts in the brains of sheep vaccinated by nasal route, in contrast to control sheep that showed the presence of brain cysts. This also followed results by El-Malky *et al.*[29] who detected a significantly lower brain cyst count following IN vaccination with *Toxoplasma* lysate antigen. Similarly, Said *et al.*[30] revealed significant brain cyst count reduction of 58.6%, together with remarkably high levels of anti-*T. gondii* antibodies (IgG, IgA) after IN immunization of mice with nano-encapsulated melatonin. Mévélec *et al.*[31] showed that the SAG1 targeting vaccine clearly

enhanced the immune response and although it significantly reduced brain parasite load it achieved a decrease of only 33%. Several studies showed that partial immunity could be induced after immunization with *T. gondii* antigens. Some mucosal immunization routes have been explored, but the nasal route is considered a potent route as it induces both mucosal and systemic responses to antigens[10,11,30].

The present work confirmed the immunoprotective effects of SAG1-loaded MNPs and the most efficient route of administration by analyzing specific anti-*T. gondii* IgG & IgA levels in sera of all immunized mice groups. The highest level of IgG was detected in the SAG1-loaded MNPs IN immunized group followed by the SAG1-loaded MNPs ID immunized group and the lowest level of IgG was in SAG1 alone ID immunized group. This conformed with Ducournau *et al*.[11] who detected a slight increase in serum IgG observed over time until it became significantly different one year after the full immunization protocol. On the other hand, Fasquelle *et al*.[32] showed no increase in the optical density of IgG antibody after three nasal administrations of maltodextrin nanoparticles containing killed *T. gondii* antigens, suggesting no triggering of the humoral immune response. Ducournau *et al*.[10] also revealed that no specific IgG was detected whatever the route of vaccine administration, while the positive control group infected with oocysts showed increased levels of IgG two months after oral infection. These inconsistencies might be due to differences in the antigen loaded on maltodextrin and the doses of the vaccination.

Regarding the level of IgA, the highest level was also detected in the SAG1-loaded MNPs ID immunized group followed by the SAG1-loaded MNPs IN immunized group and the lowest level was in the SAG1 alone ID immunized group. According to El-Malky *et al*.[29], IN administration of *Toxoplasma* lysate antigen (TLA) was able to enhance mucosal IgA production but couldn't enhance humoral immune response. On the other hand, intramuscular administration of the tested vaccine enhanced humoral Th1 response. Mévélec *et al*.[31] also revealed that the IN route alone did not induce a systemic humoral response, but when combined with the subcutaneous route production of IgG was increased. However, this contrasted with Ducournau *et al*.[11] who followed immune response to IN vaccination of squirrel monkeys (n = 48) with total extract of soluble proteins of *T. gondii* loaded on MNPs and showed a weak IgA titer after vaccination in only three monkeys.

In our study, cellular immune responses were also evaluated among all vaccinated groups by analyzing cytokine secretion levels as IL-10 and IL-12 to determine whether IN or ID route was more protective. The highest level of IL-10 was detected in SAG1-loaded MNPs IN immunized group followed by SAG1-loaded MNPs ID immunized group and the least level was in SAG1 alone ID immunized group indicating that the downregulating cytokine minimizes the exaggerated immune response to challenged infection after completion of immunization schedule. This disagreed with Ducournau *et al*.[10] who found IL-10 production was not increased in the nasal route group (140 pg/mL) but was largely greater in both groups vaccinated by intradermal routes (840 pg/ml).

Regarding the level of IL-12, the highest level of IL-12 was detected also in the SAG1-loaded MNPs IN immunized group followed by the SAG1-loaded MNPs ID immunized group and the least level of IgG in the SAG1 alone ID immunized group indicating that IN administration of the tested vaccine initiated a more protective immune response. Similarly, Ducournau *et al*.[10] detected increased levels of IL-12 following stimulation by *Toxoplasma* proteins associated with maltodextrin nanoparticles (TE/DGNP), while controls did not secrete relevant levels of this cytokine. Sang *et al*.[33] also detected significantly higher levels of IL-12 in mice immunized with either single or double DNA vaccines.

In conclusion, the utilization of potent immunogenic antigens in conjunction with nanoparticles can be a great potential for the advancement of a successful vaccine. MNPs could be used as an effective nanocarrier for SAG1, and the IN route as a needle-free method of administration for a promising effective route against toxoplasmosis. Further studies are recommended to assess the toxicity and safety concerns in the preparation of an effective vaccination against toxoplasmosis.

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Conflict of interest: The authors assert that no existing or possible conflicts of interest are associated with this manuscript's research, writing, and publication.

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