# The use of ultasonication for preparation of nano-adjuvant rabies inactivated vaccine

**Amany M. El-zieny[1], A. F. Soliman[1], S. I.El-Dek[2], and A.A.Farghali[2]**

[1]Department of Rabies , Veterinary Serum and Vaccine Research Institute.

[2] Materials Science and Nanotechnology Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt.

## Abstract:

The use of engineered nanomaterials (ENMs) that possess unique physicochemical properties in adjuvants enable researchers to achieve improved protection against infectious diseases. Recently, several studies were conducted on the use of nanoparticles as adjuvant to enhance the immunogenicity of antigen and decrease the dose of immunologic adjuvants. In this work, we synthesized aluminum hydroxide nanoparticles using sonochemical technique nanoparticles, were characterized using X-ray diffraction, high resolution transmission electron microscope and zeta size potential. The result demonstrated that the size of aluminum hydroxide nanoparticles was 51 nm in diameter that exhibited more potent antigen-specific antibody response than that of the micro-sized aluminum hydroxide gel particles (9 mm), so the prepared nano aluminum hydroxide was used as adjuvant for the inactivated rabies vaccine comparing with the traditional aluminum hydroxide gel and the dose of the prepared inactivated nana rabies vaccine was reduced, inducing high prolonged potent immune response in vaccinated dog.

**Keywords:** Adjuvant, Nano aluminum hydroxide , Sonochemical Technique, Inactivated rabies vaccine,.

## Introduction

Rabies is an acute viral encephalomyelitis caused by a virus belonging to family *Rhabdoviridae* **(Finke and Conzelmann. 2005)**. Vaccination is recommended by the World Health Organization (WHO) for control of rabies .According to the official reports, more than 60,000 person die annually from rabies, with 99% living in Asia and Africa. **(Bahloul et al., 2005 and Finnegan et al., 2002).** Rabies vaccine is used in two distinct situations that include pre- and post exposure treatments. The protective function is the production of neutralizing antibodies, either IgM or IgG, which are able to prevent the entry of virus into cells **(Knobel et al., 2005)**. In this regard, regular vaccination of animals, human rabies has controlled in Europe. **(Moore and Hanlon, 2010).**

To improve the effectiveness of rabies vaccines,in veterinary practice aluminum hydroxide is used as an adjuvant **(Lindblad, 2004)**. Although, aluminum hydroxide enhances immune response, some disadvantages were recorded including, the destructive effect on tissues (necrosis),thr induction of prolonged inflammation causing severe irritation at the site of injection, provoke of T helper

2 response only, the weak cellular immune response, and unwanted IgE reactions, restrict its application in vaccine formulations. **(Wang and Singh, 2011, Sivakumar et al.,2011 and Petrovsky and Aguilar,2004).** Therefore, new adjuvants are required to enhance the immunogenicity of weak antigens with tittle side effects, long-term immune stimulation, and simultaneous stimulation of humoral, cellular, and mucosal responses,.

One feature of nanoparticles is the capability of trapping or capturing molecules such as proteins and nucleic acids. Hence, promising methods are provided for antigen delivery to improve the function of the immune system by selection of targeting antigen-presenting cells. As regards, delivery of antigens to antigen-presenting cells, in particular, dendritic cells, and their stimulation are important issues in the development and improvement of vaccine potency. Therefore, vaccination systems based on nanoparticles create opportunities for controlled delivery of antigens to desired immune cells.**(Zhao, et al., 2014).**It was shown that nanoparticles such as silver, gold, and calcium phosphate enhance the immunogenicity of antigens. **(Dykman, et al., 2010 and Xu, et al., 2013 and He Q,et al.,2000)**.

Conventionally, nanoparticles are prepared by physicochemical techniques including chemical vapor deposition, physical vapor deposition, grinding systems, and solvothermal synthesis **(M. A. Ahme et al., 2014)**. These approaches usually need high-cost instruments, and they are performed under dangerous conditions using hazardous reagents. Some of these procedures also have complications such as instability and aggregation of the synthesized particles. Currently, green synthesis of nanoparticles has been exponentially used because of the lower cost of production as well as the simplicity of synthesis.

In the present study, we synthesized aluminum hydroxide nanoparticles using sonochemical method. The prepared nanoparticles were characterized using X-ray diffraction, high resolution transmission electron microscope and zeta size potential. Consequently the nanoparticles were used as an adjuvant for preparation of the inactivated rabies vaccine and compared to the traditional aluminum hydroxide gel based vaccine. This work was designed to reduce the dose of the vaccine to induce high prolonged potent immune response in vaccinated dog .

## Materials and Methods

### 1-Rabies virus:

BHK-21 cell culture adapted Evelyn Rokitincki Abelesth strain of rabies virus (ERA),a fixed rabies virus, was kindly supplied by Prof. P. Sureau, WHO Collaborating Centers for References and Researches in Rabies Institute Pasteur, Paris, France. The virus was provided in a lyophilized form with a titer of $10^6$ $TCID_{50}$ / ml. It was used for vaccine preparation, serum neutralization test (SNT) and preparation of virus antigen for ELISA. Challenge virus strain (CVS) is a fixed virus strain derived from the original Pasteur strain, propagated and fixed in mice brain. It was obtained from Pasteur Institute, Paris, in a lyophilized form. CVS had a titer of $10^5 MILD_{50}$/ml and used NIH during evaluation of the prepared vaccine formula. It is the legal strain used for rabies challenge in mice.

### 2.Reference Rabies Vaccine:

Delcavac Rabies vaccine was obtained from Mycofarm UK Limited, (Science Park, Cambridge, UK) . It was used as reference vaccine in application of NIH test. The vaccine contains rabies virus strain RIV/PTA/78/BHK clone 8 Batch 75894 A (ERA strain) aluminum hydroxide gel as an adjuvant.

## 3-Vaccine adjuvants:

### 3.1-Aluminum hydroxide gel:

Sterile aluminum hydroxide gel 2% was supplied by Superfos Biosector , Frydenlands Denmark and was used as adjuvant to an inactivated rabies vaccine form .

### 3.2-Nano aluminum hydroxide:

We synthesized aluminum hydroxide nanoparticles using sonochemical method. Aqueous solution of the appropriate metal nitrate; namely $Al(NO_3)_3.9H_2O$ was prepared. Sonochemical assisted co-precipitation technique **( Malyala and Singh , 2010 and S.A. Abdel Moaty et al., 2016)** was used to obtain the layered hydroxide by the conversion of metal salts into hydroxides, which occurs immediately at alkaline conditions using ultrasonic probe for dispersion in adequate amounts of deionized water. Droplets of ammonia were added on metal nitrate solution with slow rate until complete precipitation and then kept stirred in ultrasonic at 60°C for 30 minutes. The produced solutions were separated using centrifuge, thoroughly washed and finally dried at 80°C.

The obtained white powder was examined by X-ray diffraction analysis using PANalytical Empyrean model 202964 which confirmed the crystalline phase of the nanopowder by Cu-$K_\alpha$ radiation (wave length 1.54Å) at an accelerating voltage 40 kV, current of 35 mA, scan angle range from 20 to 80 and scan step $0.02_o$. The crystallite size (L) was determined from the measurements of the FWHM using Scherrer formula: $L_{XRD} = 0.9\,\lambda / (\beta \cos\theta)$; where $\beta$ is full width at half maximum of the line spectrum in radians, $\theta$ is Bragg's angle, $\lambda$ is the radiation wavelength.

The microstructure of the nanopowder samples was investigated by transmission electron microscopy JEOL JSM – 1230 (Japan) with an acceleration voltage of 200 kV

Zeta size and potential were depicted using Malvern Nano-Zs90 zeta sizer series which measures hydrodynamic diameter from below a nanometer to several microns using dynamic light scattering and the zeta potential was also recorded.

### 3.3-Adsorbance test :

This test was applied to detect the suitable required ratio of both type of adjuvant (aluminum hydroxide gel and nano aluminum hydroxide) to absorb the virus completely, using different dilutions of gel (10%, 20% .30% and 40%) and nano aluminum hydroxide (2.5%, 5% , 10% and 20%).The infectivity titer of the residual non absorbed virus was measured.

## 4- Vaccine preparation :

To prepare the virus suspension, the virus was replicated at multiplicity of infection (M.O.I), rate of 2:1 of virus/BHK-21 cells. The virus suspension was inactivated using binary ethylenamine then divided into 2 parts, where aluminum hydroxide gel was added to the inactivated virus suspension as 20% and nano aluminum hydroxide was added to the anther part at concentrations of 5%. (as the result of absorbance test).

**5-Virus inactivation by Binary Ethyleneimine (BEI):**

Rabies virus was inactivated using binary ethylenamine (BEI) where 3% concentration of BEI was added at $37^0$C to the viral suspension. The mixture was stirred continuously at $37^o$C for 3.5 hours. Inactivation process was stopped by addition of cold sodium thiosulphate with a final concentration of 2% **(WHO, 1973)**.

**6- Calculation the dose of nano aluminum hydroxide rabies vaccine :**

The dose of nano rabies vaccine was calculated by injection of different doses of the vaccine (2ml,1ml and 0.5ml) S/C in dog then calculate the titer of each dose.

**7-Experimental animals:**

**7.1-puppies:** Twenty one native breed puppies (3-month age) were used to test the safety and potency of the prepared vaccines. These puppies were found to be free from rabies antibodies as screened by serum neutralization test

**7.2-Mice**:

Two hundred and forty Albino Swiss mice (18-22g) 3-4 weeks old were supplied by the Department of Pet Animal Vaccine Research, VSVR, Abbasia, Cairo. These mice were used in the National Institute of Health, USA (NIH) test and safety of prepared rabies vaccine formulae. **(Seligmann, 1973 and Larghi and Nebel, 19 80).**

**8 . Evaluation of prepared inactivated rabies vaccines:**

**8.1. Sterility test**:

Samples from each prepared inactivated rabies vaccine formulae were cultured on Tryptose Phosphate, Thioglcolate broth, Sabouraud's agar and PPLO media according to **Code of Fedral Regulation of USA (1986).**

**8.2. Safety test:**

**1- In puppies:**

Six puppies were divided into 2 groups each one injected with double doses of each prepared vaccine (4ml from aluminum hydroxide gel vaccine and 2ml of nano vaccine), S/C in different sites and observed for 15 days for development of any clinical signs or local reaction.

**2- In Mice :**

The prepared vaccines were inoculated I/P at a dose of 0.5 ml into 40 wined mice and observed for 21 days for any signs of rabies.

**8.3. Potency:**

**8. 3.1. NIH test in mice:**

The potency of the two prepared vaccine formulae was evaluated using National Institute of Health (NIH) test to determine their antigenic value.

The NIH test was carried out according to **(Seligmann ,1973)**. NIH test was carried out using the volumetric method to evaluate antigenic value (AV) as follow:

$$\text{Antigenic value (AV)} = \frac{ED_{50} \text{ of reference vaccine}}{}$$

$ED_{50}$ of test vaccine

**8.3.2. Serological evaluation in puppies:**

Experimental puppies were divided into 5 groups (3 animals / each) and were immunized subcutaneously as follow:

**Group-1**: vaccinated with 2ml of inactivated rabies vaccine adjuvanted with aluminum hydroxide gel.

**Group-2:** vaccinated with 0.5ml of inactivated rabies vaccine adjuvanted with aluminum hydroxide gel.

**Group-3:** vaccinated with 2ml of inactivated rabies vaccine adjuvanted with nano aluminum hydroxide.

**Group-4**: vaccinated with 0.5ml of inactivated rabies vaccine, adjuvanted by nano aluminum hydroxide.

**Group-5**: was kept without vaccination as control.

**8.3.2.1. Serum neutralization test (SNT):**

It was carried out according to (**18) (Rossiter et al., 1985),** for sero-conversion of vaccinated and control puppies to rabies antibodies. The antibody titer was calculated as the mean of the reciprocal of final serum dilution which neutralized and inhibited the cytopathic effect (CPE) after 3 days of 100 $TCID_{50}$ of rabies virus.

**8.3.2.2 Indirect ELISA:**

The indirect ELISA was carried out for quantitative determination of rabies antibodies according to **(Cliquet et al., 2003).**

## Results and discussion

X-ray diffraction pattern of the prepared nanoparticles is illustrated in Fig. (1). X-ray diffraction analysis has identified the aluminum hydroxide adjuvant as crystalline aluminum oxyhydroxide, AlOOH. The pattern pointed to the formation of fine crystallites as the peaks are broad and with small intensities. The data are compared and indexed with the ICDD card no 00-015-0136 and 00-029-0041 as $Al(OH)_3$ with hexagonal and monoclinic structure respectively.

**Fig. (1) XRD pattern of $Al(OH)_3$ nanoparticles**

The high resolution transmission electron micrographs are presented as in Fig.2 (a, b). The micrographs clarified the ultra fine size of the synthesized nanoparticles which was 51 nm in diameter as in fig. (2,b) and the distinguished homogeneity. The selected area electron diffraction assured the good crystallinity and the co-existence of two crystal symmetry as each ring consists of double dots coinciding with each other that were 10 nm, as in Fig. (2, a), HRTEM micrograph and SAED pattern of the nanoparticles.

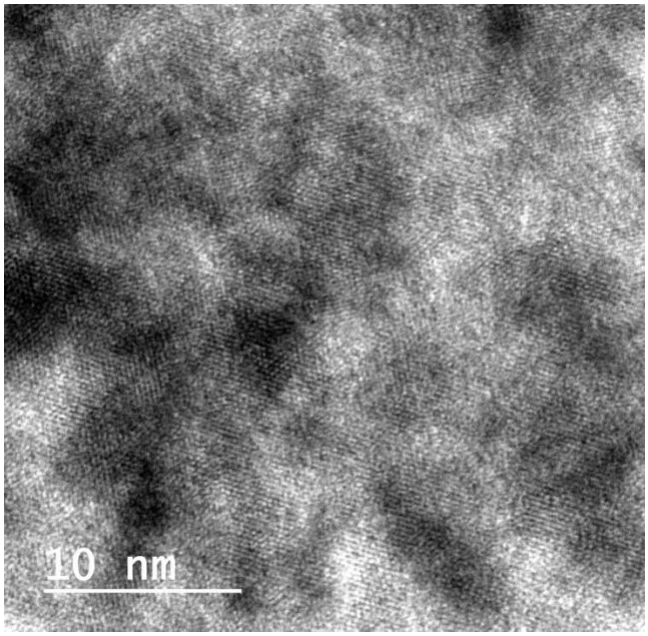
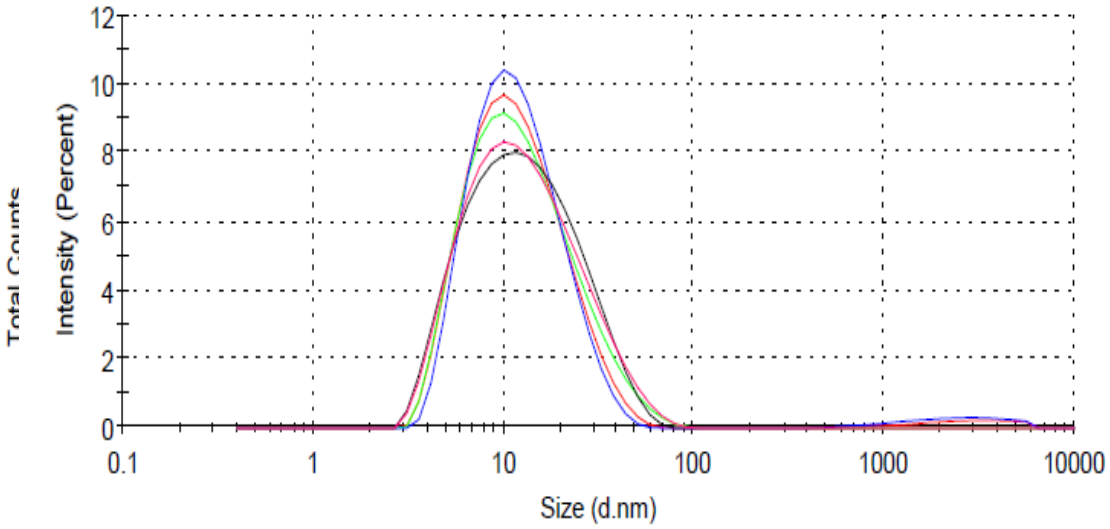



**Fig.( 2: a):** HRTEM micrograph. Crystallinity and crystal symmetry by high resolution transmission electron micrographs

The zeta potential was measured to examine the stability of the nanoparticles in deionized water as in figure (3 a, b). The values are positive and large 60




mV pointing to the high stability of the nanoparticles in this solution for a long period. The hydrodynamic diameter is depicted by Dynamic light scattering technique and found to be around 13 nm for 5 times successively

Fig. (3:a,b): Zeta potential and size of the prepared nanoparticles in deionized water

**Table (1) : Result of Aluminum hydroxide gel absorbence test**

| Dilution of alhydragel | Residual virus titer ($log_{10}$ $TCID_{50}$/ml) |
|---|---|
| 10% | 3.4 |
| 20% | Less than 1 |
| 30% | 0 |
| 40% | 0 |

**Table (2 ) : Result of nano aluminum hydroxide absorbence test**

| Concentration of nano aluminum hydroxide | Residual virus titer ($log_{10}$ $TCID_{50}$/ml) |
|---|---|
| 2.5% | 3.4 |
| 5% | Less than 1 |
| 10% | 0 |
| 20% | 0 |

The results of adsorbance test of both aluminum hydroxide gel and nano aluminum hydroxide are shown in tables (1&2 ) was revealed that 30% and 40% dilution resulted in complete virus adsorbance while at dilution of 10% and 20% residual virus titer was to $10^{3.4}$ and less than 1 $TCID_{50}$/ml respectively and by different concentrations of nano aluminum hydroxide \ (2.5%, 5% , 10% and 20% ) it was found that 10% and 20% dilution resulted in complete virus absorbance while at concentration of 2.5% and 5% residual virus titer reached $10^{3.4}$ and less than 1 $TCID_{50}$/ml respectively.

The obtained result of titration to calculate the dose of nano rabies vaccine, indicated that the dose of 0.5 ml from nano rabies vaccine was protective with $10^6$ titer

`

Table (3)**:** Sterility and safety of the prepared adjuvants

| Type of vaccine | Sterility | | | Safety/Potency | | |
|---|---|---|---|---|---|---|
| | Bacterial | Fungal | Mycoplasma | Mice | Dog | NIH potency in mic |
| AL(OH)3 GEL | -ve | -ve | -ve | Safe | Safe | 2.1 |
| Nano AL(OH)3 | -ve | -ve | -ve | Safe | Safe | 2.6 |

Table (4): Mean titers of rabies serum neutralizing antibodies in sera of vaccinated dogs

| Animal Group | Tested vaccine Formulae | Used Dos | Mean rabies serum neutralizing antibody titer^ on periods post vaccination |
|---|---|---|---|

| | | | WPV* | | | | MPV* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | L(OH)3 | 2ml | 2 | 8 | 16 | 32 | 32 | 64 | 64 | 64 | 64 | 64 | 64 | 64 |
| | | 0.5ml | 2 | 4 | 8 | 16 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| | ano AL (OH) 3 | 2ml | <2 | 16 | 32 | 64 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| | | 0.5ml | 2 | 8 | 16 | 32 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 |
| | vaccinated | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

***** WPV: Weeks Post Vaccination * MPV: Months Post Vaccination

Group-1 &2: vaccinated with inactivated rabies virus vaccine adjuvanted with $AL(OH)_3$

Group-3 &4: vaccinated with inactivated rabies virus vaccine adjuvanted with nano$Al(OH)_3$.

Group-5: unvaccinated animal as control.

♦WPV= week post-vaccination

^Antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of rabies virus.

N.B: The protective level of rabies antibody titer is (32).

The vaccination of dogs with the two prepared inactivated rabies vaccines formulae evoked different levels of specific rabies antibodies as measured by SNT and ELISA as demonstrated in tables **(5&6) .**The protective level of neutralizing antibody titers against rabies virus was (32) as reported by **(Sikes et al., 1971).**The level of rabies serum neutralizing antibody titers in third and fourth groups (vaccinated with 2ml and 0.5 ml) were high during the 4th week after vaccination with nana aluminum rabies vaccine as 32 and 64 respectively. The level of antibodies reached the highest values on the 2nd month post vaccination which were 128 and 64 as compared to group-1 & 2 (puppies receiving aluminum hydroxide gel vaccine, 2ml &0.5 ml respectively ) in which the antibody titer was 64 and 32 at the end of the experiment. These titers appear to be higher than the recommended protective level (32).These results are parallel to and confirmed by the finding of **(Bass et al., 1982).** In addition, the use of ELISA for the quantification of rabies antibody titers in post vaccinated animals was established by **(Servat et al., 2007),** as in table**(6).** From the obtained results, it was clear that the inactivated rabies vaccine adjuvanated with nano aluminum hydroxide was potent with a dose of 0.5 ml

**Table (5): Mean titers of rabies serum antibodies in sera of vaccinated dogs measured by ELISA**

| Animal Group | Tested vaccin Formulae | sed Dose (m | Mean anti-rabies antibody titer ($log_{10}$/ml) on periods post vaccination | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | WPV* | | | | MPV^ | | | | | | | |
| | | | 1 | 2 | 3 | 4 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | AL(OH)3 | 2 | 0.5 | 0.7 | 1.1 | 1.6 | 2.1 | 2.1 | 2.1 | 2.1 | 1.95 | 2.1 | 2.0 | 1.95 |
| 2 | | 0.5 | 0.3 | 0.5 | 0.8 | 1.2 | 1.4 | 1.8 | 1.9 | 1.95 | 1.9 | 1.95 | 1.80 | 1.80 |
| 3 | Nano AL (OH)3 | 2 | 0.7 | 1.0 | 1.5 | 1.8 | 2.1 | 2.11 | 2.2 | 2.1 | 2.2 | 2.1 | 1.8 | 1.95 |
| 4 | | 0.5 | 0.3 | 0.8 | 1.2 | 1.4 | 2.0 | 2.3 | 2.31 | 2 | 2 | 1.95 | 1.85 | 1.80 |

***** WPV: Weeks Post Vaccination ^ MPV: Months Post Vaccination
Group-1 &2: vaccinated with inactivated rabies vaccine adjuvanted with $AL(OH)_3$
Group-3 &4: vaccinated with inactivated rabies vaccine adjuvanted with nano $Al(OH)_3$.
Group-5: unvaccinated animal as control.
♦WPV= week post-vaccination

Currently, synthesis of nanoparticles by ultra sonication technique has been used because of the lower cost of production as well as the simplicity of synthesis.
In this study, the adjuvanticity effect of $Al(OH)_3$ nano particles on the rabies veterinary vaccine was assessed and the results were compared with commercially inactivated rabies vaccine containing aluminum hydroxide gel adjuvant. The results showed that the size of particles was 50–55 nm as in figure (1,2&3)). In addition, XRD results that confirmed the identity of synthesized nano $Al(OH)_3$. .The results demonstrate the shape, crystallinity, hydroxyl content and size of nano aluminum hydroxide adjuvant, that have an important role in increasing antibody titers of vaccinated animals as shown in table (5&6), decrease the dose of vaccination as immunologic adjuvants, as in table (2).
The titer of nanoparticles was determined by absorbance test and NIH test as in table (2&3).The potent adjuvant activity of the aluminum hydroxide nanoparticles was likely related to their increasing the surface area and ability to more effectively facilitate the uptake of the antigens adsorbed on them by antigen-presenting cells. The defined physicochemical properties of AlOOH nanoparticles that synthesized by ultra-sonication technique, with determining crystal structure, hydroxyl content ,particle shape and size which could capable of NLRP3 inflammasome activation and stimulating IL-1β production in THP-1 cells and BMDCs **(Sun et al., 2013)).** Finally, the local inflammation induced by aluminum hydroxide nanoparticles in the injection sites was milder than that induced by microparticles. Simply reducing the particle size of the traditional aluminum hydroxide adjuvant into nanometers represents a novel and effective approach to improve its adjuvanticity**(Li X et al**., **2014)**.

## Conclusion

From the obtained results, it was concluded that the adjuvant activity of the synthesized aluminum hydroxide nanoparticles by ultra-sonication technique was more potent as compared with the traditional aluminum hydroxide gel microparticles, leadindg to reduce the dose of the vaccine and induce high prolonged potent immune response in vaccinated dog with inactivated rabies vaccine.

## References

Bahloul C, Taieb D, Kaabi B, et al.( 2005) :Comparative evaluation of specific ELISA and RFFIT antibody assays in the assessment of dog immunity against rabies. Epidemiol Infect.;133(4):749–757.

Bass, R., P. Gunzel, D. Henschler, J. Konig, D. Lorke, D. Neurbert, E. Schutz, D. Schuppan & G. Zbinden: LD50 versus acute toxicity. Arch. Toxicol. 1982, 51, 183–186.

Cliquet, F.; Muller, T.; Mutinelli, F.; Geronutti, S.; Brochier, B.; Selhorst, T.; Schereffer, J.L.; Karfft, N.; Burow, J.; Schameitat, A.; Schluter, H. and

Aubert, M. (2003):Standardization and establishment of a rabies ELISA test in European laboratories for assessing the efficacy of oral fox vaccination.Vaccine 18 (28): 3272-3279

Code of Federal Regulation of USA (1986): Animal and animal by product, 9\1986, published by the office of federal register national archives and record administration.
Dykman LA, Staroverov SA, Bogatyrev VA, Shchyogolev SY. (2010) :Adjuvant properties of gold nanoparticles..;5(11–12):748–761.

Finke S, Conzelmann KK.( 2005): Replication strategies of rabies virus. Virus Res.;111(2):120–131.

Finnegan CJ, Brookes SM, Johnson N, et al.( 2002) :Rabies in North America and Europe. J R Soc Med.;95(1):9–13.

He Q, Mitchell AR, Johnson SL, Wagner-Bartak C, Morcol T, Bell SJ. (2000) :Calcium phosphate nanoparticle adjuvant. Clin Diagn Lab Immunol.;7(6):899–903.

Knobel DL, Cleaveland S, Coleman PG, et al. (2004) :5Re-evaluating the burden of rabies in Africa and Asia. Bull World Health Organ.;83(5):360–368.

Larghi, O.P. and A.E. (1980):Nebel, Rabies virus inactivation by binary ethylenimine: new method for inactivated vaccine production. J Clin Microbiol,. **11**(2): p. 120 -130

Li X, Aldayel AMand Cui Z.**(2014):**Aluminum hydroxide nanoparticles show a stronger vaccine adjuvant activity than traditional aluminum hydroxide microparticles**.** Jan 10;173:148-57. doi: 10.1016/j.jconrel. 10.032. Epub.

M. A. Ahmed, S. F. Mansour, S. I. El-dek, S. M. Abd-Elwahab, M. K. Ahmed (2014)**:** Synthesis, sintering and characterization of porous nano-structured CaP bioceramics prepared by a two-step sintering method Ceramics international, 40, 8, Part B, (2014), 12807-12820

Malyala P and Singh M. (2010): Micro/nanoparticle adjuvants: preparation and formulation with antigens. In: Davies G, editor. Vaccine Adjuvants: Methods and Protocols. Totowa, NJ: Humana Press;. pp. 91–101.

Moore SM, Hanlon CA. (2010): Rabies-specific antibodies: measuring surrogates of protection against a fatal disease. PLoS Negl Trop Dis.;4(3):e595.

Petrovsky N, Aguilar JC. (2004) :Vaccine adjuvants: current state and future trends. Immunol Cell Biol.;82(5):488–496.

Rossiter et al., (1985), Rossiter P.B, Jessett D.M and Taylor W.P.(1985): Microneutralization system for use with different strains of peste des petits ruminants virus Top. Anim. Hlth. Prod., 17 (2):75-81.

S.A. Abdel Moaty, A.A. Farghali and Rehab Khaled (2016): Preparation, characterization and antimicrobial applications of Zn-Fe LDH against MRSA.Materials Science & Engineering C 68(2016) 184-193
Seligmann, B. (1973): The NIH test for potency. LaboratoryTechniques in rabies.3rd Ed. WHO monograph series, (23) Chapter 33:279.

Servat, A.; Feyssaguet, M.; Blanchard, I.; Morize, J.L.; Schereffer, J.L.; Boue, F. and Cliquet, F.A. (2007):A quantitative indirect ELISA to monitor the effectiveness of rabies vaccination in domestic and wild carnivore. J.Immunol. Methods 2007; 318: 1-10

Sikes, R.K., Peacock, C.V.lAcha, P.,Arko, P.J and Dierksm R(1971): Rabies vaccines: Duration of immunity, a study in dogs. J.A.V.M.A., 158(11):1491-1499.

Sivakumar SM, Safhi MM, Kannadasan M, Sukumaran N. (2011) :Vaccine adjuvants – current status and prospects on controlled release adjuvancity. Saudi Pharm J.;19(4):197–206.

Wang W, Singh M. (2011) :Selection of adjuvants for enhanced vaccine potency. World J Vaccines.;1(2):33–78. 1

WHO (1973): Expert Committees on Rabies. 6th report Technical Report Series No. 523: 29-50

Zhao L, Seth A, Wibowo N, et al. (2014) : Nanoparticle vaccines. Vaccine.;32(3):327–337.

Xu Y, Tang H, Liu JH, Wang H, Liu Y.( 2013) :Evaluation of the adjuvant effect of silver nanoparticles both in vitro and in vivo. Toxicol Lett.;219(1):42–48.

## الملخص العربي

## استخدام التفتيت المتناهي لتحضير لقاح السعار المثبط المحمل علي جزيئات متناهية الصغر

**د. اماني محمد عباس د.أكرم فؤاد سليمان[1] ا. د. سماء امام الدق[2] د. احمد علي احمد فرغلي[2]**

**[1] معهد بحوث الامصل واللقاحات البيطرية ، العباسية ، القاهرة , [2] كلية الدراسات العليا –جامعة بني سويف**

ان استخدام مواد النانو المهندسة التي تمتلك خصائص فيزوكميائية فريدة في تحضير المحفزات المناعية تمكن الباحثين من تحقيق تحسين الحماية ضد الأمراض المعدية ويعتبر مرض السعار من الأمراض المعدية شديدة الخطورة علي الانسان و الحيوان. وفي الآونة الأخيرة أجريت عدة دراسات عن استخدام جسيمات النانو كمحفزات مناعية وذاك لتعزيز القوة المناعية للانتيجين وإنقاص جرعة المحفزات المناعية. وفي هذا العمل، تم تحضير جسيمات نانو هيدروكسيد الألومنيوم باستخدام تقنية ultrasonication والتي تم التعرف عليها باستخدام حيود الأشعة السينية والميكروسكوب الإلكتروني وحجم زيتا. وكانت النتيجة تغير حجم جسيمات هيدروكسيد الألومنيوم من 10 ميكروميتر في القطر الي 51 نانوميتر وقد استخدم نانو هيدروكسيد الألومنيوم المحضر كمحفز مناعي adjuvant في تحضير لقاح السعار المثبط بدل من جيل هيدروكسيد الألومنيوم التقليدي والهدف من ذلك لتقليل جرعة اللقاح و احداث استجابه مناعيه قوية للكلاب المحصنة بلقاح السعار المثبط.