Trails of use of nano-particles as adjuvant for inactivated bovine viral diarrhea vaccine

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1. Abstract

Bovine viral diarrhea virus (**BVDV)** with different genotypes considered as one of the most important viruses in genus *Pestivirus* at animal level is consider an ideal research model for hepatitis C virus of the same genus of *Flavivirus*. Many traditional inactivated BVDV vaccine have been used adjuvanted by Alhydragel or oil. The current study focused on formulation anew inactivated BVDV vaccine using innovated nano material (meso porous nano silica) prepared with as 9 parts of viral suspension: 1- Part of dissolved adjuvant. Sterility and safety of the adjuvant alone and with the prepared vaccine containing two genotypes of BVDV (1and 2) and potency test on in vaccinated calves revealed that such adjuvant and vaccine are free from foreign contaminants and the vaccine is potent provided calves with protection against BVDV coverage a period of 9 month for both genotypes using two doses of 1.5mL of both inactivated virus vaccine. This study recommended other complementary study to clarify the detail keeping quality issues and field study.

Key words: BVDV, meseporous nano silica, vaccine , potency**.**

2. Introduction

BVDV recently and from past has great economic and endemic infectious importance in either Egypt or worldwide. The most danger point is immune suppressive effect on affected animal so it called sometimes animal AIDS [5]. It has different infectious from mild to watery hemorrhagic fatal diarrhea. In case of genotype 2 infection, abortion and persistent animal hidden spreader disease represent the most dramatic point of strength of viral existence [7]**.** BVDV belongs to family ***Faviviridae*** genus *Pestivirus* with (border disease, classical swine fever and hepatitis c virus). The virus has genetical three type with different subtype (BVDV-1, BVDV-2 and BVDV-3 what is called Hobi like pesti virus. There is antigenic relationship and cross reactivity between virus types [12,3]**.** Regarding Egypt, inactivated BVDV is included in the inactivated Egyptian pnemo-4 vaccine which provides a coverage

protection period against BVDV in vaccinated calves for about 6 months. Such vaccine is adjuvanted with Alhydragel with very restricted effect on cellular immunity and contain type-1 only [16]**.** Meso pore nano silica (MSNPs) as patent nano particle has many biological uses such as antioxidant and antigen carrier and vaccine delivery [2] which allows nanoscale switches to gain precise control over the release of cargo from these inorganic–organic mesostructured materials. Regardless of the delivery platform chosen, the functions and properties of each scaffold can be modulated, or tuned, to adapt to the circumstances of the infection, or disease, through the use of integrated systems within the context of applications-driven chemistry [19]**.**

So, the present study was designated to test antigen carrying character of MSNPs with BVDV and its immunologic reaction in vivo measuring by monitoring the levels of induced antibodies in vaccinated calves and follow up the provided protection period.

3. Materials and Method

1. Viruses

Egyptian local strain of Bovine viral diarrhea virus genotype-1 (BVD-1) (Iman strain) and reference BVD-2 (125 strain) were propagated and tittered on Madin Darby Bovine Kidney (MDBK) cell culture which has been proved to be free of any infectious agents especially non cytopathic strain of BVD virus. These viruses were supplied by Rinderpest Like Diseases Research Dept (RLDRD), Veterinary Serum and Vaccine Research Institute Abbasia, Cairo (VSVRI). And used in vaccine preparation and SNT according to [4].

2. Lab animals:

2.1. Mice :

Fifteen Swiss Albino weaned mice of 4 weeks old were used to test the safety of MSNPs and the prepared vaccine (5 mice for MSNPs; 5mice for the vaccine and 5 mice kept as control) in accordance to [11].

2.2. Calves

Twelve calves (Native breed and 4 weeks age) were found to be free from BVDV antibodies as screened by SNT and used for evaluation of the prepared vaccine potency in accordance to [11].

3. Tissue culture:

Madin Darby Bovine Kidney (MDBK) cell culture was supplied by VSVRI and used for BVDV propagation; virus titration and serum neutralization test in accordance to [11].

4. Virus inactivation:

Harvested and titrated BDV suspension was inactivated using 1% ascorbic acid at 37º C for 24 hours according to [9].

5. Vaccine preparation:

5.1. Preparation of mesoporous silica particles (MSNPs):

MSNs were produced and characterized according to to [1] Briefly, 1g of Cetylpyridinium bromide hydrate **(**CPB) and

0.6 g of urea were dissolved in 30 ml of deionized water with continuous stirring for 30 min. Cyclohexane (30 ml) and isopropanol (1.2 ml) were added to the solution. Under strong stirring at room temperature, TEOS (2.7 ml) was gently added dropwise to the mixed solution in 5 min. After 30 min of sturdy stirring, the reaction mixture was heated to 85 °C and then kept for 17 hours at 4° C. The mixture was centrifuged and thoroughly washed with acetone and water several times. MSNs were left to dry for 12 hrs. at room temperature and then annealed at 600 °C for 6 h in air to remove the surfactant template. The resulting material was characterized using Field emission scanning electron microscopy Quanta FEG (FESEM)

5.2. Scanning electron micrographs:

It was carried out for monitoring and confirm carrying efficacy of nano particle and viral particle **at Materials Science and Nanotechnology Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Sueif University** [19,6].

5.3. Vaccine formulation:

Nine parts of each inactivated virus fluid were mixed together with one part of nano powder dissolved in tris HCL 7.6-7.8 pH according to [19,6].

6. Quality control of the prepared vaccine:

Quality control testing of the prepared vaccine including freedom of foreign contaminants; safety and potency was performed in accordance to [11] **.**

7. Potency test in calves:

Twelve male calves were divided into 4 groups (three calves/ group) as follow:

Group (1): was intramuscularly vaccinated with 1ml/calf of vaccine formula MSNPs by two injections with 2 weeks interval

Group (2): was vaccinated in the same manner as group-1 using a vaccine dose of 1.5ml/ calf

Group (3): was vaccinated n the same manner as group-1 using a vaccine dose of 2ml/ calf

Group (4): was kept as non-vaccinated contact control group all in accordance to [11]**.**

8. Serum neutralization test (SNT):

For monitoring of the induced BVD antibody titers in vaccinated calves, SNT was performed on MDBK cell line according to **Fulton et al (1995**) and the antibody titer was calculated as neutralization index (NI) according to[14,15],The test was carried out on serum samples obtained from all calf groups on month intervals up to 10 months post vaccination

9. Enzyme linked immunosorbent assay (ELISA):

It is carried out according to [17] on the same collected serum samples.

4. Results

Figure (1) showed the electron microscopic picture of nano particle and theory of increasing the functional surface [1].**Tables (1 and 3)** and **figure (2)** showed immunizing efficacy of the prepared BVDV adjuvanted by MSNPs using serum neutralization test and confirmed by ELISA applied on collected serum sample from

inoculated calves with 1ml of prepared vaccine formula revealing neutralization index clarified protection coverage period of 5months when reached minimal protective level (0.9) for both types of BVDV (1 and 2) that showed a weaker efficacy in comparative to already registered locally produced vaccine pnemo-4 which give 6 month protection period under field condition [13,16]. **Tables (2 and 4)** and **figure (3)** showed NI in collected serum samples from inoculated calves with 1.5ml of prepared vaccine formula showing a protection coverage period of 9 months reaching the minimal protective level (0.9) for the both types of BVDV antibodies in agreement with what reported by [15,13] that showed a weaker efficacy in comparative to already registered locally produced vaccine pnemo-4 which give 6 month protection period under field condition [15,8] **Table (5 and 6)** showed that results of calves 'vaccination with a dose of 2ml of inoculation induced nearly similar SNT results obtained by the use of 1.5 ml inoculated dose and have no economic significance to use**.** This result when compare with that obtained by using the gel adjuvanted vaccine a positive developed aspect at point of reduced the dose volume from 2 to 1.5 with longer protection period (from 6 to 9 months) the thing which could be attributed to developed adsorbance area and release properties of nano material [6,19].

5. Discussion

Bovine viral diarrhea virus is one of important flavivirus member genus *Pestivirus* together with one of most important human viruses; Hepatitis c virus; and consider a model for its properties especially at nano-technique level study. It is enveloped of one large RNA open reading frame [10]. From its economical and epizootically importance, many international and local preparations of combined vaccine trails were carried out and already have been produce and use, such as cattle master -4 and pneuo-4 with other pneumonia viruses such as bovine herpes, parainflunza and bovine respiratory syncytial viruses [16].In this study we tried to produce specific bivalent bovine viral diarrhea vaccine by using a new nano adjuvant of Mesoporous silica to provide long duration of immunity in vaccinated calves with a trail to minimize the protective dose. Mesoporous silica nano particles (MSNPs) with large surface area and pore volume can serve as efficient carriers for various therapeutic agents such as vaccine adjuvant purpose. The functionalization of MSNPs with molecular, supramolecular or polymer moieties, provides the material with great versatility such as performing vaccine adjuvant delivery tasks, which makes the delivery process highly controllable. This emerging area at the interface of chemistry and the life sciences offers a broad palette of opportunities for vaccine development researchers with interests ranging from sol–gel science, the fabrication of nanomaterials, supramolecular chemistry, controllable drug delivery and targeted theranostics in biology and medicine[19].Both of type 1 and 2 of bovine

viral diarrhea viral suspension was prepared and bivalent vaccine was prepared after inactivation of virus by 1% ascorbic acid [9]. One common approach to modify characteristics, such as solubility, drug release capability, adsorption of drugs, and specific targeting of silica-based drug delivery systems is to combine the properties of silica with those of other organic or inorganic materials. The functionalization of a silica surface with organic groups is a relatively simple process which can be achieved either by co-condensation during its synthesis. We prepare the mixture on Tris alkaline range from 7.6 to7.9 A diversity of organic functionalities ranging from the relatively simple alkyl [2]. Polymeric nano-capsules or single crystals surrounded by a porous silica shell have also been employed. Furthermore, this kind of core/shell strategy has been recently employed to provide stability to micellar nanoparticles, where the outer silica shell prevents the micelles from breaking down upon dilution [4] MSNPs as adjuvant in ratio 10% was prepared with viral suspension with different dose of inoculation in calves as potency test for the prepared vaccine after good sterility and safety tests applied according to [11]. Figure (1) showed the electron microscopic picture of nano particle and theory of increasing the functional surface **[1]. Tables (1 and 3)**and **figure (2)** showed immunizing efficacy of the prepared BVDV adjuvanted by MSNPs using serum neutralization test and confirmed by ELISA applied on collected serum sample from inoculated calves with 1ml of prepared vaccine formula revealing neutralization index clarified protection coverage period of 5months when reached minimal protective level (0.9) for both types of BVDV(1 and 2)that showed aweaker efficacy in comparative to already registered locally produced vaccine pnemo-4 which give 6 month protection period under field condition[16,13]**. Tables (2 and 4)** and **figure (3)** showed NI in collected serum samples from inoculated calves with 1.5ml of prepared vaccine formula showing a protection coverage period of 9 months reaching the minimal protective level (0.9) for the both types of BVDV antibodies in agreement with what reported by [13,16] that showed a weaker efficacy in comparative to already registered locally produced vaccine pnemo-4 which give 6 month protection period under field condition ([16,7]**. Table (5 and 6)** showed that results of calves 'vaccination with a dose of 2ml of inoculation induced nearly similar SNT results obtained by the use of 1.5 ml inoculated dose and have no economic significance to use**.** This result when compare with that obtained by using the gel adjuvanted vaccine a positive developed aspect at point of reduced the dose volume from 2 to 1.5 with longer protection period (from 6 to 9 months) the thing which could be attributed to developed adsorbance area and release properties of nano material [6,19].

6. Conclusion

We recommended the usage of safe potent long duration BVDV **MSNPs** adjuvanted inactivated vaccine to protect calves against BVDV infection through the use of 2 doses of 1.5 ml 2weeks interval.

7. References

1-AbouAitah K.; A.A. Farghali, A. Swiderska-Sroda, W. Lojkowski, A.-F. Razin,M. Khedr (2016): pH-controlled release system for curcumin based on functionalized dendritic mesoporous silica nanoparticles, J. Nanomed. Nanotechnol. (07)351

2-Abou Aitah K.; A. Swiderska-Sroda, A.A. Farghali, J. Wojnarowicz, A. Stefanek,S. Gierlotka, A. Opalinska, A.K. Allayeh, T. Ciach, W. Lojkowski (2018): Folic acid-con-jugated mesoporous silica particles as nanocarriers of natural prodrugs for cancer targeting and antioxidant action, Onco target 9 (41) (2018) 26466–26490

3-Chen, Y.; Liu, H.; Huang, Q.; Meng, M.; Xia, H. Wu (2020): Evaluation of protection against bovine viral diarrhea virus type 2 after vaccination of the calves with bovine virus diarrhea virus type 1 combo inactivated vaccine. Arq. Bras. Med. Vet. Zootic. Vol. 72, No .3,2020.

12-4-Fulton, R.W., Confer, A.W,; Burge, L.J.; Porino, L.J; D'Offay, J.M.; payton , M.E. and Mock , R.E.(1995); Antibody viral vaccine containing BHV, BVD, PI-3 and BRSV immunogens and subsequent revaccination at day 140. Vacccines, 13: 725-733.

4-Huo Q.; J. Liu, L.-Q. Wang, Y. Jiang, T. N. Lambert, E. Fang, J. Am. (2006):
Chem. Soc.2006, 128, 6447.

5-Izedin goga, Kristaq Berxholi, Beqe Hulaj, Driton Sylejmani, Boris Yakobson and Yehuda Stram (2014): Genotyping and phylogentic analysis of bovine viral diarrhoea (BVDV) isolates in kosova, veterinaria italiana 2014, 50 (1), 69-72.

6-Liu, Z., Ru, J., Sun, S., Teng, Z., Dong, H., Song, P., ...&Guo, H. (2019). Uniform dendrimer-like mesoporous silica nanoparticles as a nano-adjuvant for foot-and-mouth disease virus-like particle vaccine. Journal of Materials Chemistry B, 7(21), 3446-3454.

7-Maha Raafat (2007): Serosurveillance of Bovine Viral Diarrhea Genotype in Breeding Farm Animal in Egypt, **V. M. Sci. Thesis**, Benha University Faculty of Veterinary Medicine, Virology Department.

8-Maha Raafat (2011): studies on pneumogen -5 vaccine which containing viruses causing diarrhea and respiratory symptoms in cattle, **PH Sci. Thesis**, Benha University Faculty of Veterinary Medicine, Virology Department.
Egypt. J. Agric. Res., 95 (3), 2017 1349

9-ABD EL FADIL, MAHA RAAFAT, RASHAEL HAWARY andEFFAT L. EL-SAYED(2017): Validity of ascorbic acid as viral inactivant for infectious bovine rhinotrachities virus and bovine viral diharrhea virus. Egypt. J. Agric. Res., 95 (3), 2017 1349.

10-Mostafa Ahmed El-gaffary,ostafa Mahoud bashandy , Alaa Rafaat Ahmed

,Ola el Borady(2020) : Self-assembled gold nanoparticle for in-vitro inhibition of bovine viral diarrhea virus as Surrogte Model for HCV. Heliyon e04045.

11-OIE (2019): Manual of diagnostic tests and vaccines for terrestrial animals P.109-122.

12-Peletto S, Zucco F, Pitti M,Gobbi Emarco LD, Caramelli M, asoero L, Acutis PL. (2012): Detection and phylogentic analysis of an atypical pesti virus and strain IZSPLV, Res. Vet Sci. 201292(1|):147-150.

13-Rasha,I.EL-Hawary and Hanaa A. Mostaf (2016): Immunological response of locally prepared oil adjuvanted pneumo-5 vaccine in calves . journal of veterinary medical research ,2016, 24 (1):complete

14-Reed, L. J. and Muench, A. (1938): A simple method of estimating fifty percent and points. Amer. J. Hyg., **(1938).** 27: 443-445.

15-Samira, S.T.; El-Sabbagh, M.M.A. and Allam, A.M.M. (2009): Preparation of multivalent inactivated against some bovine respiratory viruses adjuvanted by Nigella sativa oil and its evaluation in pregnant Buffaloes and their calves). Global Veterinarian (2009) (6): 429-433.

16-Samira, S.T.; El-Sabbagh, M.M.A. and Ghaly, H.M. (2019): Preparation of combined inactivated BVD, IBR, PI3 and respiratory syncytial virus (BRSV) J. Egypt. Vet. Med. Ass. 61, no. 4: 251-263.

17-Voller, A., Bidwell, D.E. and Annbarlett, M. (1976): Enzyme immune assays in diagnostic medicine, theory and practice. Bull. World Health, organ, **(2001)**: 63: 55-65.

18-World organization for animal health (2019): Tests for sterility and freedom from contamination of biological materials intended for veterinary use in: World organization for animal health, editor. 2019. Paris.

19-Zongxi Li, **Jonathan C. Barnes**, **Aleksandr Bosoy** , **J. Fraser Stoddart and Jeffrey I. Zink (2012):** Mesoporous silica nanoparticles in biomedical applications. *Chem. Soc. Rev.*, 2012, **41**, 2590-2605

Table 1: Mean BVD serum neutralizing antibody index in calves inoculated with 1 ml of formulated vaccine with MSNPs

Periods post vaccination	Mean BVDV-NI against		
	BVDV-1	BVDV-2	Control
0 day	0.0	0.0	0.0
1stMPI*	1.56	1.6	0.0
2ndMPI	1.39	1.44	0.0
3rdMPI	1.2	1.3	0.0
4thMPI	1.05	1.16	0.0
5thMPI	0.9	1.0	0.0
6th MPI	0.75	0.85	0.0
7th MPI	0.6	0.7	0.0
8th MPI	0.45	0.55	0.0
9th MPI	0.37	0.4	0.0

*MPI= month post first inoculation

Table 2: Mean ELISA antibody titer of BVDV in calves inoculated with 1 ml of formulated vaccine with MSNPs

Periods post vaccination	Mean BVDV-ELISA antibody titer (log10)		
	BvDv-1	BvDv-2	Control
0 day	0.06	0.08	0.0
1stMPI*	1.8	1.8	0.0
2ndMPI	1.6	1.62	0.0
rdMPI3	1.4	1.45	0.0
4thMPI	1.23	1.3	0.0
5thMPI	1.05	1.12	0.0
6th MPI	0.9	0.99	0.0
7th MPI	0.75	0.94	0.0
8th MPI	0.6	0.8	0.0
9th MPI	0.47	0.65	0.0

***MPI**=month post first inoculation

Table (3): Mean BVD serum neutralizing antibody index in calves inoculated with 1.5 ml of formulated vaccine with MSNPs

Periods post vaccination	Mean BVDV-NI		
	BvDv-1 NI	BvDv-2 NI	Control
0 day	0.0	0.0	0.0
1stMPI*	2.2	2.21	0.0
2ndMPI	2.06	2.06	0.0
3rdMPI	1.9	1.92	0.0
4thMPI	1.72	1.75	0.0
5thMPI	1.58	1.6	0.0
6thMPI	1.35	1.38	0.0
7thMPI	1.2	1.21	0.0
8thMPI	1.04	1.o9	0.0
9thMPI	0.92	0.95	0.0

***MPI=** month post first inoculation

Table (4): Mean ELISA antibody titer of BVDV in calves inoculated with 1.5 ml of formulated vaccine with MSNPs

Periods post vaccination	Mean BVDV-ELISA antibody titer (log10) against		
	BVDV-1	BVDV-2	Control
0 day	0.04	0.01	0.0
1stMPI*	2.3	2.3	0.0
2ndMPI	2.18	2.19	0.0
3rdMPI	2.0	2.02	0.0
4thMPI	1.8	1.82	0.0
5thMPI	1.65	1.7	0.0
6thMPI	1.48	1.48	0.0
7thMPI	1.3	1.31	0.0
8thMPI	1.14	1.19	0.0
9thMPI	1.02	1.05	0.0

***MPI**=month post first inoculation

Table (5): Mean serum neutralizing antibody index of both BvDv genotype in calves inoculated by 2 ml of the prepared vaccine adjuvanted by (MSNps)

	Mean BVDV- antibody index against		
Periods post vaccination	BvDv-1 NI	BvDv-2 NI	control
0 day	0.0	0.0	0.0
1stMPI*	2.25	2.3	0.0
2ndMPI	2.11	2.15	0.0
3rdMPI	1.98	2.0	0.0
4thMPI	1.8	1.88	0.0
5thMPI	1.67	1.69	0.0
6thMPI	1.5	1.55	0.0
7thMPI	1.3	1.31	0.0
8thMPI	1.15	1.2	0.0
9thMPI	0.99	1.05	0.0

***MPI**=month post first inoculation

Table (6): Mean ELISA antibody titer of BVDV in calves inoculated with 2 ml of formulated vaccine with MSNPs

Periods post vaccination	Mean BVDV-ELISA antibody titer (log10) against		
	BVDV-1	BVDV-2	control
0 day	0.0	0.0	0.0
1stMPI*	2.45	2.5	0.0
2ndMPI	2.3	2.35	0.0
3rdMPI	2.1	2.2	0.0
4thMPI	1.8	2.0	0.0
5thMPI	1.65	1.85	0.0
6thMPI	1.5	1.55	0.0
7thMPI	1.35	1.4	0.0
8thMPI	1.2	1.25	0.0
9thMPI	2.0	1.15	0.0

***MPI**=month post first inoculation

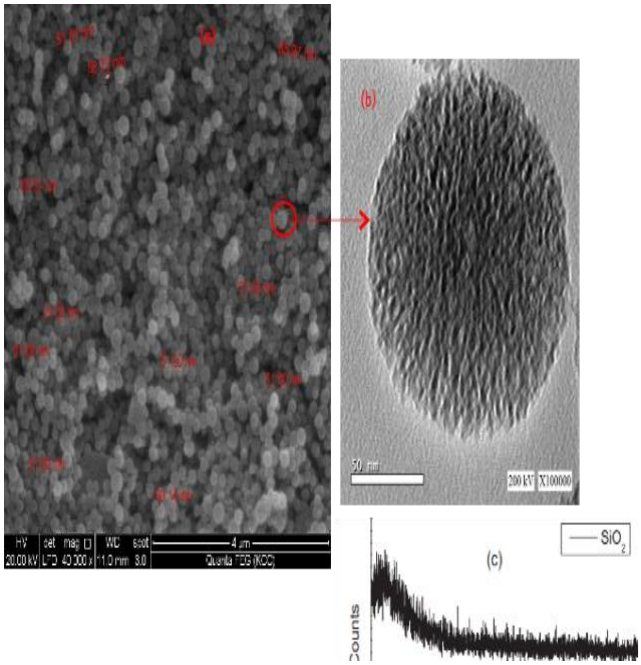

Fig (1): Scanning electron micrographs (1: a, b) illustrated the FESEM of the prepared mesoporous silica at two different magnifications. It is clear that the particles are of spherical shape with nearly equal diameters. Another striking feature is their homogenous distribution. Increasing magnification to get closer to the surface, we observed the porous nature of the particles prepared hand in hand with the roughness. Fig (1.c) assure the amorphous nature of the powder as a broad hump is seen.

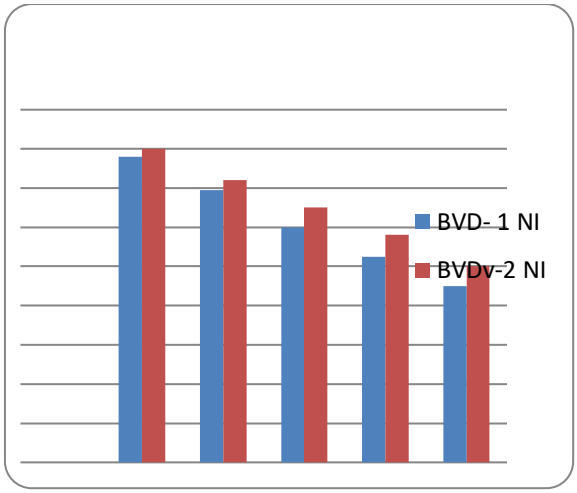

Fig (2): Mean BVD serum neutralizing antibody index in calves inoculated with 1 ml of formulated vaccine with MSNPs

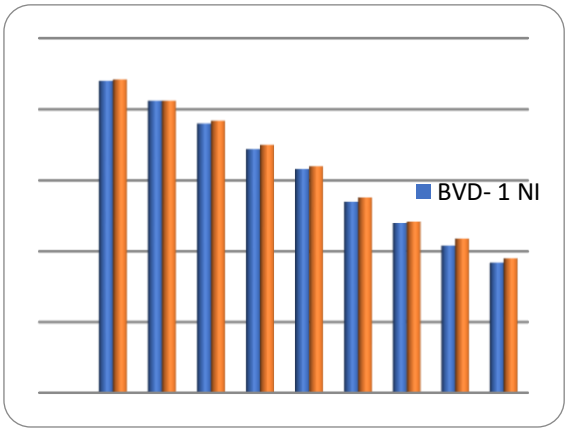

Fig (3): Mean BVD serum neutralizing antibody index in calves inoculated with 1.5 ml of formulated vaccine with MSNPs