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ANTIVIRAL ACTIVITY EFFECT OF SILVER NANOPARTICLES (AgNPs) SOLUTION AGAINST THE GROWTH OF INFECTIOUS BURSAL DISEASE VIRUS ON EMBRYONATED CHICKEN EGGS WITH ELISA TEST

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Abstract

Infectious bursal disease virus is one of the strategic infectious disease in Indonesia. Despite disinfection and vaccination technology has been doing, the cases still frequently occur and it needs another alternative technology to be developed to against IBD virus. This research try to answer the problem, it examines the effect of antiviral activity of silver nanoparticles (AgNPs) solution against the growth of infectious bursal disease virus in embryonated chicken eggs with ELISA Test. The research has two methods, the first method is conducted by mixing a solution of AgNPs and IBD Virus, two hours before inoculated (preventive method) and the second method is the virus inoculated first, 48 hours later the AgNPs solution injected (therapy method). Each method has several dosage of AgNPs solution respectively 0 ppm (positive control 20 ppm, 40 ppm, and 50 ppm). Virus samples taken from the *choriallantoic* membrane (CAM) and the embryo by crushed method. Results based on the value of OD (optical density) ELISA Test and Statistical Test ANOVA General Linier Models Univariate with Post-Hoc Duncan 5%, both methods have no significant difference ($p>0.05$), it means the solution of AgNPs has good preventative and therapeutic characteristic. The mean of OD values also showed dosage of 20ppm is most effective dosage in against the growth of the virus, the dosage has significant difference ($p<0.05$). The decreasing amount of virus in CAM and in embryos were not significantly different ($p>0.05$), in both CAM and embryo AgNPs solution has good antiviral properties.

Keywords: Silver Nanoparticles, Antiviral, Infectious Bursal Disesase, ELISA, Embryonated Chicken Eggs.

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1. INTRODUCTION

Globalization development is in line with the population growth, life style changes nutritious awareness and better education degree as well as the increasing of farming product order such as egg, meat and milk (Delgado et all, 1999). The chances to run farming agribusinesses open widely because Indonesia has great human and natural resources. The future farming industry that free from infectious diseases (diseases that very pathogenic with high economic risk) is the main goal of farming (Riady, 2005).

One of the strategic infectious diseases on poultry farm sector is Infectious Bursal Disease (IBD), generally called gumboro. IBD virus is very infectious and spread easily by contact directly or through water, feces and contaminated feed (Budinuryanto, 2000).

Technology development has invented nanomaterial as new antivirus agent, silver nanoparticles (AgNPs) has researched as anti microba and also proof active against some kinds of viruses including HIV virus (Human Immunodeficiency Virus), Hepatitis B, herpes simplex virus, respiratory syncytial virus and monkey pox. The researchers said that silver can attack various location target on the virus part and there may be lower resistance compared with conventional antivirus (Galdiero, 2011).

Based on the above theory, it is necessary to do the research on strategic infectious disease on chicken, one of them is IBD to know the potency of silver nanoparticles solution as antiviral on the chicken diseases.

2. METHOD AND RESULT PRE RESEARCH

2.1. Synthesis and characterization PSA

Synthesis AgNPs solution use chemistry reduction method, then dilutioned on 10ppm, 20 ppm and 50 ppm using aquabides as the solvent. The result of PSA characterization show that spreading silver scale 10 nm as much as 0,5%, the most 50,75 nm and 58,77 nm each as much as 9,2%.

2.2. Toxicology Test Figure. 1(b)

Toxicology test is done on 15 TAB Figure 1(a) and 1(b) Synthesis AgNPs at the age 9 – 11 days injected AgNPs solution use chemistry reduction method solution through chorioallantoic membrane (CAM), then seen the percentage of hatching number of TAB. The percentage of hatching number reach 73,3%. Two TAB are very weak and

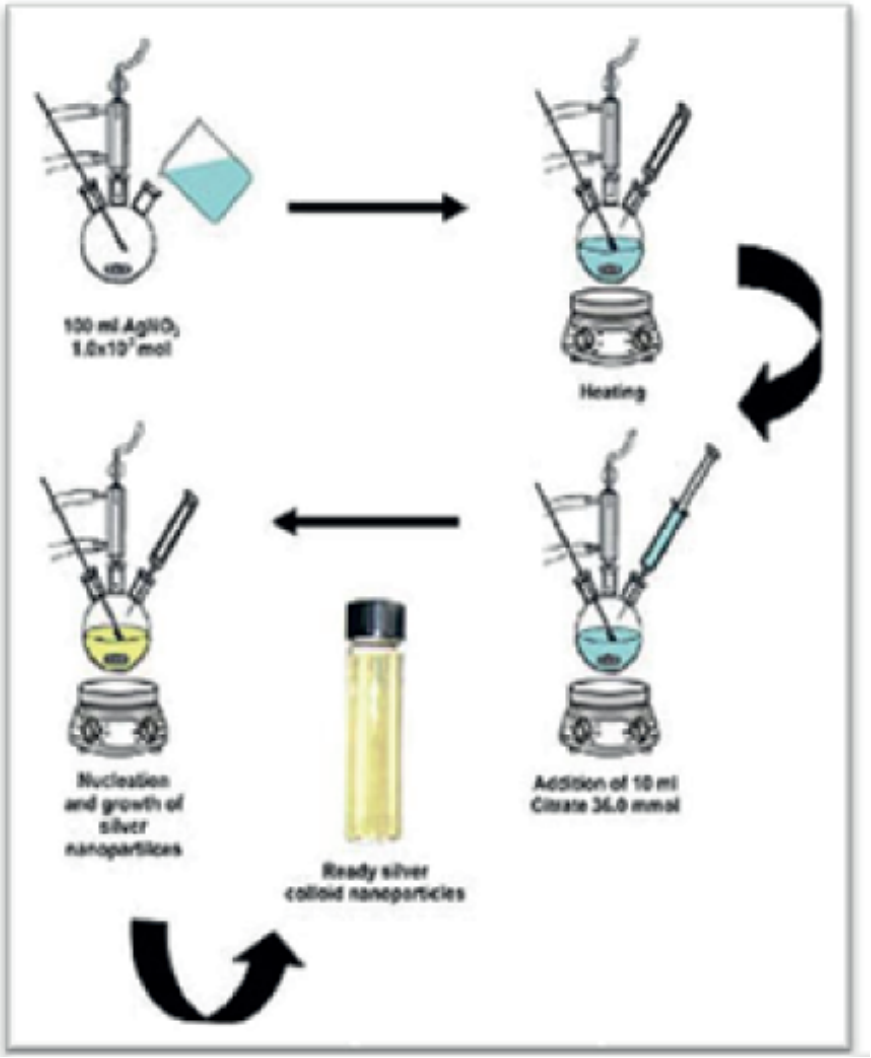

Figure 1

dead one day after injected and two others grow well but they have difficulty to hatch. It can be concluded that AgNPs don't have significant toxic activity on TAB because TAB don't dead specifically and homogenious.

2.3. Antiserum production

IBD antiserum production on chicken of 3 weeks old and rabbit of 4 months old, vaccinated for 3 months with 5 times rehearsal. Antiserum is collected two weeks after the latest vaccination, then tested chequerboard ELISA optimum to know the best dilatation antibody.

2.4. Multiply Virus

Multiply virus is done by inoculated virus suspense on TAB. The multiply IBD virus 10 TAB of 9–11 days old, then injected virus suspension into the 10 TAB through chorioallantoic membrane (CAM) with 0,1 ml/TAB dosage. It is done 3 timer per phase on some virus then tested on chequerboard ELISA optimal to take sample with optimum number virus.

2.5. Optimization (chequerboard) ELISA

Optimization Chequerboard is done to determine antigen and antibody that will be used in the research and optimum dilatation from each antigen, antibody, conjugate as well as substrate that will be used. (Suwarno dkk, 2010)

Based on the value average result OD (Optical Density) chequerboard ELISA optimal test, it can be concluded that good dilatation is group 1. That was antibody and antigen dilatation 1/50, that had low OD Blank value while OD sample value is high. The sample will be used is the optimum OD sample got from IBD strain Georgia active vaccine with three times phases.

2.6. Virus Titration

Virus titration is done to know the EID 50 virus in each virus suspension. Virus titration is done by dilatation multiple 10 begun from 10^{-1}; 10^{-2}; 10^{-3} etc until 10^{-11} then continued by inoculate virus on TAB from 10^{-1} dilatation until 10^{-8} dilatation, each 4 repetiton. After that the virus is collected by souring the CAM and tested ELISA. The result OD ELISA value on titration phase is counted s/p value and known the proportion titration positive reaction and next counted virus EID_{50} value by using spearman-karber pattern.Based on the spearman-karber pattern it is got the virus titer of $10^{2.75}$ $EID_{50/ml.}$

3. RESEARCH METHOD

This research has been done in virology and immunology veterinary laboratorium of Faculty of Veterinary Medicine and PSA AgNPs solution test in physic solid material F MIPA of ITS from November 2015 untill Mei 2016.

The research material that be used are 10 days embryonated eggs as many as 95 got from chicken farm In Mojokerto area. Silver nanoparticles solution (AgNPs) on 10

Table 1: The result Positive Negative treatment based on S/P value.

Method	Dosage AgNPs	Treatment	CAM	Embryo
Control + Method I	0 ppm	P0	+	+
Treatment Group Method I	10 ppm	P1	+	-
	20 ppm	P2	-	-
	50 ppm	P3	+	-
Control + Method II	0 ppm	P4	+	+
Treatment Group Method II	10 ppm	P5	+	-
	20 ppm	P6	-	-
	50 ppm	P7	-	-
Control -	PZ	P8	-	-

ppm, 20 ppm and 50 ppm that synthesized using chemistry reduction method, sodium chlorid (NaCL) physiological sterile, IBD virus $10^{2,75}$ EID $_{150/ml}$ derived from active vaccine PT Indovax product distributed by PT Otasindo Prima Satwa Jakarta, alcohol and test material indirect Double Antibody Sandwich (ELISA).

Tools in use in this research are microcenttifuge tube, Erlen Meyer, Microplate ELISA, the observer, dental drill/nail, pencil, 1 ml squirt, scotch tape, mortar, and stamper, incubator, pipet, pinset, egg tray, micropipette, sentrifus, scissors, cotton, auto clave and bulb.

4. THE RESEARCH PLAN

This research used complete random plan by factorial then tested by Analysis of Variance (ANOVA) and real honest differ test (Duncan) Data analysis is to compare among three factors treatment. They are method factor (method I) IBD virus and silver nanoparticle solution contacted outside TAB and incubated on room temperature for two hours then inoculated on TAB through CAM / preventive method and method II the IBD virus injected on TAB through CAM, incubated on 37°C, after 48 hours silver nanoparticle solution injected on TAB through CAM on the same hole/therapy/threat method. Dosage factor (10 ppm, 20 ppm, 50 ppm)and location factor of sampling (CAM and embryo) used General Linear Model univariate analysis.

5. RESULT AND DISCUSSION

The counting result positive and negative sampling based on s/p value from average OD sampling as served in table 2. It showed that the total virus decrease on treatment group in method I (P1, P2, P3) as well as in method II (P5(a,b), P6 (a,b), P7(a,b)) to positive control that is (Po(a,b)and P4(a,b)) seen based on average OD value. It means that AgNPs solution have virusidal activity as well as therapy. Based on ANOVA test (Analysis of Variance) SPSS Windows 20 showed that there is no real difference between method I and method II. It means that AgNPs solution has good virusidal as well as therapy.

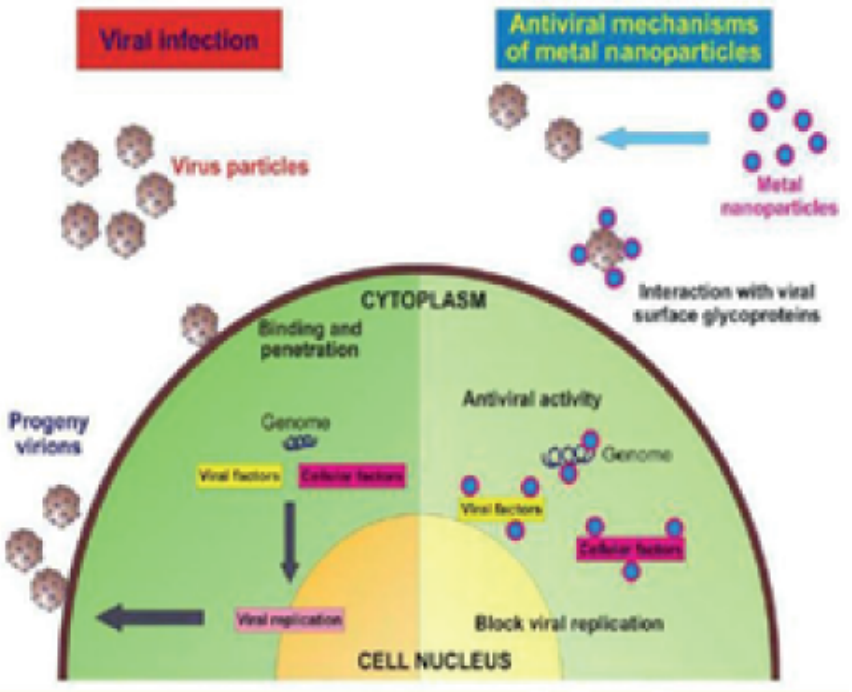

Figure 2: Schematic model of a virus infecting an eukaryotic cell and antiviral mechanism of metal nanoparticles. *Source: Galdiero, 2011.*

The success of method I that has virucidal is in line with some theories about mechanism of action from AgNPs work to prevent penetration between virus and host cell so it has virusidally and good for disinfectant, including: interaction with gp120 on HIV-1 virus, work competitively hamper bonding virus to cell on HSV-1 virus, inactivated virus particles before enter to Tacaribe virus (Galdiero et al.,2011).

The success on method II that has therapeutic properties also linear with previous research, They are on HIV-1 AgNPs solution also hamper phase after the entry of HIV-1 (Castro and Haghi, 2012), on Mechanism of action from AgNPs to Hepatitis B virus that is AgNPs interact with DNA double strain virus, bound virus particles to hamper replication and inactivate virus (Galdiero et al., 2011), the result of Pedersen research,

Table 2: The average OD value based on dosage.

Dosage	OD Value
20 ppm	1,835[a]
50 ppm	1,906[ab]
10 ppm	1,981[b]
0 ppm	2,214[c]
Note: The difference superscript on one column show the real difference (p<0,05)	

(2014) done to Ebola virus, and also AgNPs antiviral activity after the virus infection on HSV-1 and HPIV-3 have decrease replication virus consistently (Gaikwad et al.,2013).

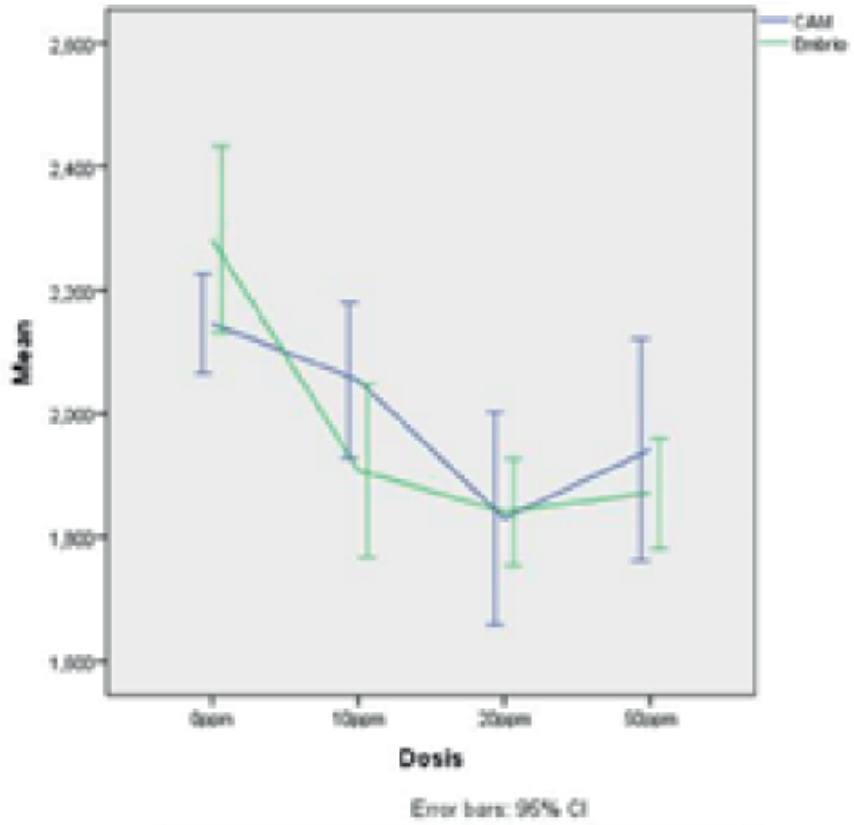

Figure 3: Dosage chart against OD values based on CAM and embryo.

The result shows that dosage factor is real different (p<0,05). 20ppm dosage is more effective than 10ppm dosage in hamper the virus growth, linear with the theory of previous research both on AgNPs virucidal activity to H6NI virus by Epidemiological Research Tean of Animal Health Research Institute Council Of Agriculture Executive Yuan and On monkey pox done by Rogers et al., (2008) where the higher dosage given the stronger antiviral activity from AgNPs.

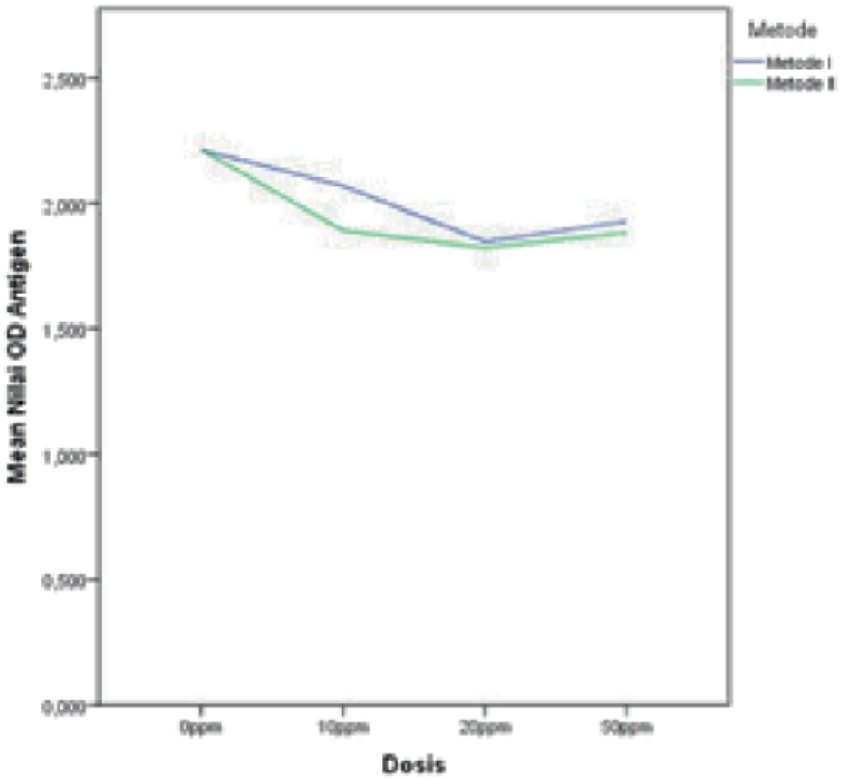

Figure 4: Dosage chart against OD values based on Methode I and II.

On 50ppm dosage have decrease antiviral power on both methods. It closely related with the influence of storage time to AgNPs solution stability so it is easy to have agglomeration on high concentration because the distance between particles become nearer. Agglomeration causes nanoparticles bounded and form bigger size (Ariyanta dkk, 2014).

The result show that there are not any real difference between decrease virus number on CAM and virus number on embryo ($p>0,05$), it means AgNPs solution work against virus as good as on CAM and embryo.

There are some other notes from result in this research. As known that IBD virus has no envelope, where no envelope virus is more stability and bear to environment changes (Machdum, 2009), while silver nanoparticles on 1-10nm size bounded easily with virus because it suits with surface scale gp120 on envelope virus (Elechiguerra et al., 2005) as the researchon HIV-1 virus, AgNPs is very virusidal. It is suggested on the next AgNPs influence research on IBD virus and other non envelope virus to add time of incubation virus mixing and AgNPs for more than two hours to get maximum virusidatily.

6. CONCLUSION

1. Silver nanoparticles solution with 10nm diameters has antiviral ability to the growth IBD virus on TAB.
2. Silver nanoparticles solution is virusidality and therapy on IBD virus.
3. Concentration silver nanoparticles solution most effective against IBD virus is 20ppm
4. The influence antiviral activity silver nanoparticles solution to IBD virus on chorio Alantoic Membrane as good as activity silver nanoparticles on embryo.

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