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EXAMINATION OF THE IMMUNOGENICITY OF EXPERIMENTAL SUBUNIT VACCINE AGAINST NEWCASTLE DISEASE VIRUS

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The of immunogenicity of the experimental subunit vaccine against Newcastle Disease virus was studied in a biological assay on 180, Issa Brown chickens, (80 chickens of 21 days old, and 100 chickens of 11 days old) and 50 Hybro chickens of 21 days old. The experimental subunit was prepared from purified glycoprotein subunits isolated from experimental subunit vaccine was prepared from purified glycoprotein subunits isolated from the outer envelopes of Newcastle Disease viruses, La Sota strain, which had a hemagglutinating titre of 128 HJ/0,1 ml. Antigens were adsorbed on the adjuven Al (OH)3, dissolved in 100 mmol/l PBS. Two groups of 22 and 25 experimental Issa Brown chickens, were immunized with 0,2 ml of the subunit vaccine i/m per animal, and revaccinated on day 21 of the assay. The group of 22 experimental chickens was artifically infected on day 28 after the vaccination with 200 00 EID of the virulent strain of the Newcastle Disease virus - Hertz 33 (i/m per animal). Survival percentage after the artifical infection was 95,4%. The geometric mean titres of the HI antibodies against the Newcastle Disease virus (Gmt log 2/25 ml) in the sera of the first group of 22 vaccinated chickens: were as follows: on day 7 after the vaccination 2,80, on day 21 - 2,70 and on day 28 - 4,0, and in the sera of the second group of vaccinated chickens on day 7-2,87, on day 21-2,78,and on day 28 - 4,17. The second two groups of 18 and 15 experimental Issa Brown chickens served as the negative and positive controls in the assay. The other part of the examination was carried out in a biologycal assay on chicken. Of the Hybro breed, 21 days old.Two groups of 16 and 14 experimental chickens immunized in the described way, were artificially infected after 21 days with 200 000 (the first group) and 400 000 (the second group) EID50 of the Hertz 33 strain (i/m per animal). Survival after the artificial infection was 94% in the first group, and 74% in the second group. Two other groups of 10 Hybro chickens served as the negative and positive controls in the assay. A comparative study

of the subunit vaccine and of the TB-Mukteswar vaccine was carried out inaa biologycal assay on two groups of 50 experimental Issa chickens Brown, 11 days old. Survival after the artificial infection with 200 000 EID50 of NDV, Hertz 33 strain (i/m per animal) in the chickens vaccinated with subunit vaccine was 100% white in the chickens immunized with TB-Mukteswar vaccine survival was 80%.

Key words: Newcastle disease virus, glycoprotein antigens, subunit vaccine, immunization, artificial infection, chickens.

INTRODUCTION

With regard to the importance that immunoprophylaxis plays in the control of infection with Newcastle Disease virus (a disease that still poses an acute problem in veterinary pathology) not only in our country but in many other countries, as well we would like to give a brief outline of some investigations regarding the possible application of subunit vaccines for active immunization of poultry. Previous experimence concening immunoprophylaxis of atypical fowl plague, published in numerous papers as described by Alexander, Ed. by Kluver, Ac. Publ. 1988. has shown that the existing live - attenuated and inactivated vaccines have merits and demerits, which, in many cases, prevent the successful control of Newcastle Disease.

Attenuated vaccines, prepared from live, attenuated Newcastle Disease viruses (mesogenic and sometimes lentogenic strains), generally induce protective immunity in vaccinated poultry, but often produce undesirable reactions in the form of mild or severe respiratory syndromes or low egg yield in laying hens (Nemarnik 1988.). Under certain conditions (multiple passage through susceptible organisms) there is a possibility of reactivation, as the attenuated virus from the vaccine can regain its virulence.

The defects of inactivated vaccines are: a longer period from the time of administration to the development of immunity, as well as a weaker immunogenic effect that in attenuated vaccines, due to the high level of polyclional immune response, which results in immunosuppression.

This is why more and more attention has lately been paid to the investigation of a possible application of subunit vaccines, prepared from purified glycoprotein antigens isolated from the complete viral particle and without the nucleocapsid with infective RNA and pyrogen.

The isolation of immunologically significant glycoprotein antigens from the complete particle of the Newcastle Disease virus in a purified form, with preserved biological activities (hemmaglutinating and fusional), represents a prerequisite for the preparation of a good quality immunogen which would elicit a stronger immune response in the immunized animals and which would enable us to direct immune mechanisms of the body specifically against the virus that penetrates the organism (Rey et al., 1985; Scheid et al., 1974 and 1972.).

The objective of our study was to check immunogenicity of the experimental subunit vaccine against Newcastle Disease, prepared in the Department of

Microbiology. The Faculty of Veterinary Medicine, Belgrade, in a biological assay on chickens.

MATERIAL AND METHODS

I Vaccine: The subunit vaccine was prepared from the La Sota outer envelope of Newcastle Disease virus, which had a hemagglutinating titre of 128 HJ/0,1 ml and had been adsorbed on the adjuvant Al (OH)3 dissolved in 100 mmol/l, phasphate buffered saline (PBS) (according to the method of Milić et. al.; 1991. and Milić, 1993.).

Purified glycoprotein antigens were identified in the vaccine with the SDS-PAGE method Laemmly, 1970., Gordon, 1983.). Gordon, 1983.) and HI test (Clarke and Casal, 1958; Mihaijlović; 1984.).

The direct hemagglutination method Mihajlović, 1984; Allan and Gough, 1974. Clarke and Casals, 1958.) and the method for the determination of total protein concentration Lowry et al., 1951.) helped to ascertain that the vaccinesample contained 0,170 mg/ml proteins i. e. 0,034 mg proteins in one administered dose of 0,2 ml with a hemagglutinating activity of 128 HJ/0, 1 ml.

The virus for the preparation of the subunit vaccine was propagated in the allantochorionic cavity of 11 - day old chicken embryos, for 72 hours, at the temperature of 36^{0}C. The titre of the propagated virus was log=10-9,3 EID 50 (EID 50=10-9,3/0,1ml), whilst the hemagglutinating titre was 512 HJ/0,1 ml.

II Biological assay with experimental chickens 1. The assay was done on 80 Issa Brown experimental chickens, 21-days old, and 50 Hybro chickens of the same age. Two groups of 22 i 25 Issa Brown experimental chickens were immunized with 0,2 ml of the subunit vaccine i/m per animal, and revaccinated on day 21 of the assay. Then the first group of 22 experimental chickens was artificially infected on day 28 after vaccination with 200 000 EID 50 of the Newcastle Disease virus, virulent strain Hertz 33 (i/m per chicken). All the experimental chickens had been free from HI antibodies against the Newcastle Disease virus. In order to monitor the humoral immune response with the HI test, we took blood samples from the immunized chickens on day 7,21, 28 and 35 after the vaccination.

Two other groups of nonvaccinated Issa Brown chickens served as the control groups. One of them, consisting of 18 chickens, was artificially infected with 200 000 EID 50 of the virulent strain Hertz 33 (i/m per chicken), whilst the other group, of 15 chickens, remaind nonvaccinated and not infected throughout the assay.

2. Two groups consisting of 16 and 14 Hybro experimental chicks, 21 - days old, and free from the HI antibody for the Newcastle Disease virus, were immunized in the already described manner and artificially infected on day 21 after the vaccination: one with 200 000 EID 50, the other with 400 000 EID 50 virulent strain of the Hertz 33 virus (i/m per chicken).The antibody titre in the blood serum samples experimental chickens was determined with the HI test on days 21 and 28.

Two control groups, each of 10 chickens of the same breed, were used as negative and positive controls (as in the previous assay).

3. A comparative study of our subunit vaccine with the TB - Mukteswar vaccine of the Veterinery Institute, Zemun was performed in a biological assay on two groups of 50 experimental chicks each. Issa Brown breed, 11-days old. One group was vaccinated with 0,2 ml of the subunit vaccine i/m per unit; the second group in the same way with the TB- Mukteswar vaccine (mesogenic viral strain). The HI antibody titre in the sera of the vaccinated chickens was monitored using the HI test. Both groups, were examined, 42 days after giving the Hertz strain, Newcastle. Disease virus (i/m per chicken). In addition, we examined the humoral immun response of the group containing 20 experimental Issa Brown chickens, immunized with the subunit vaccine in the above descibed way, 6 and 12 months after the vaccination.

4. The HI test was carried out according to the standard method in Limbro microplates with 0,5% suspension of chicken erythrocytes (Mihajlović, 1984; Allan and Gough, 1974; and Clarke and Casals, 1958.). The HI antibody titres in the sera of the examined chickens were expressed in mean metric titres (Gmt log 2/25 1), according to Sjurin et al., (1984).

5. The artificial infection of the experimental chickens with the virulent strain of the Hertz 33 virus, was done according to Ph. Brit (Vet.), 1985. Add., 1992. and Alexander, 1988.

RESULTS

1. Biologycal assay on experimental Issa Brown chickens, 21 days old The mean geometric titres of the HI antibodies against the Newcastle Disease virus (Gmt log 2/25ml), detected by the HI test in the sera of the two groups of 22 and 25 experimental Issa Brown chickens, on day 7 after the vaccination were 2,80 and 2,87, on day 21 0 2,70 and 2,78, on day 28 - 4,0 and 4,17, and on day 35 - 3,7 (Figures 1 and 2). The first experimental group consisting of 22 chickens was immunized with a dose of 0,2 ml of the subunit vaccine per animal, i/m; revaccinated on day 21 of the assay and artifically infected on day 28 after the vaccination with 200 000 EID50 of the Newcastle Disease virus virulent strain Hertz 33 i/m, per chicken. The survival percentage after the artificial infection, without any symptoms of the disease, was 95,4% (Figure 1).

All the nonvaccinated and all chickens artificially infected with 200 000 EID50 NDV Hertz 33 strain, (i/m per animal), died during a period of 3 - 5 days (including the positive control of 18 chickens).

The experimental group of 15 nonvaccinated and not infected, 21 - day old chickens, served as the negative control in the experiment.

On the basis of the results obtained from the biological assays of immunization followed by an artificial infection of the experimental chickens (Figures 1 and 2), it could be concluded that the tested subunit vaccine is immunogenic, i. e. it provides solid protection to the vaccinated chickens from an infection with atypical fowl plague.

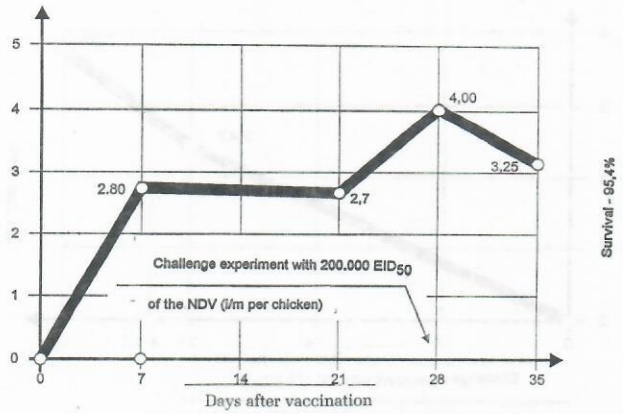

Figure 1. The mean geometric titres of HI antibodies against NDV (GMT log 2/25 ml)

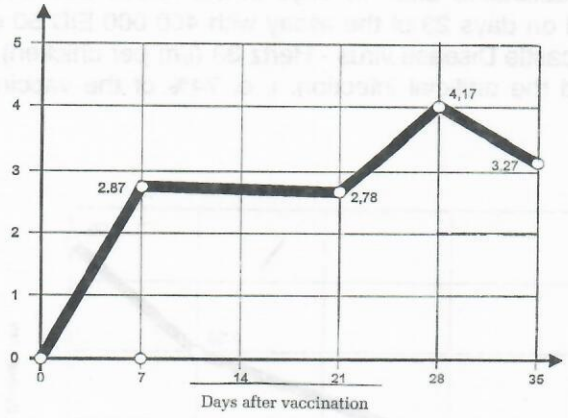

Figure 2. The mean geometric titres of HI antibodies against NDV (GMT log 2/25 nl)

2. Biologycal assay on experimental Hybro chickens, 21 days old. The subunit vaccine immunogenicity was tested by a biologiical assay on 50 Hybro chickens, 21 days - old, during one production cycle. One group, consisting of 16 chickens, vaccinated with 0,2 ml of the subunit vaccine on day 21 after birth, and revaccinated after 21 days, was artificially infected after 7 days (23 rd day of the assay) with 200 000 EID50 of the Hertz 33 strain (i/m per chicken) (Figure 3). The survival after the artificial infection was 94%; i. e. 14 chickens survived the challenge experiment without any symtomps of Newcastle Disease. The geometric mean titres of the HI antibodies against Newcastle Disease (Gmt log 2/25 ml) in the blood sera of the chickens are shown in Figure 3.

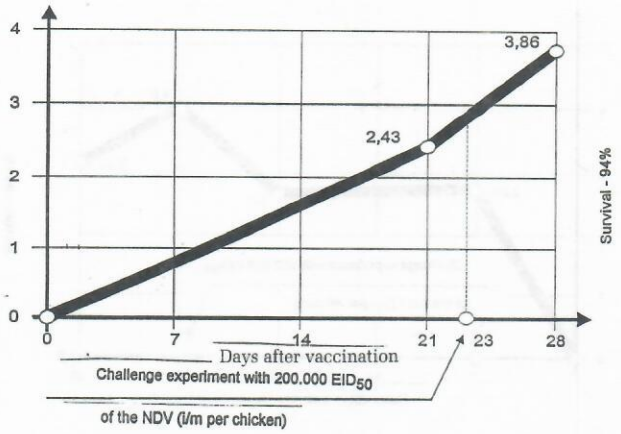

Figure 3. The mean geometric titres of HI antibodies against NDV (GMT log 2/25 ml)

The second group, of 14 Hybro chickens, vaccinated on day 21 after hatching and revaccinated after 14 days in the above described way, were artificially infected on days 23 of the assay with 400 000 EID 50 of the virulent strain of the Newcastle Disease virus - Hertz 33 (i/m per chicken), A total of 10 chickens survived the artificial infection, i. e. 74% of the vaccinated animals (Figure 4).

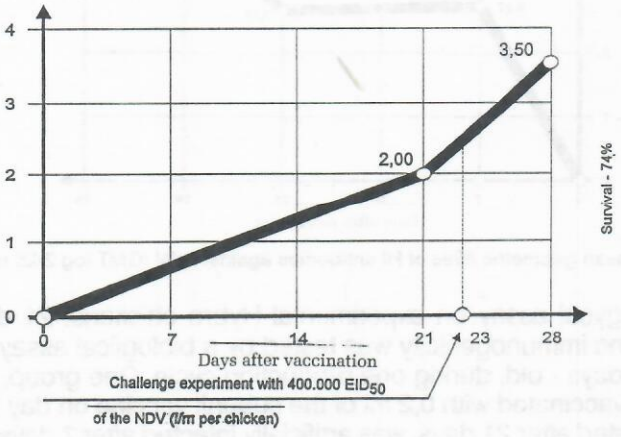

Figure 4. The mean geometric titres of HI antibodies against NDV (GMT log 2/25 ml)

The group of 10 Hybro chickens nonvaccinated and artificially infected (of the same age) with 200 000 EID 50 of the Hertz 33 strain (i/m per animal) died in 3 - 5 days from Newcastle Disease.

The fourth group, consisting of 10 nonvaccinated and not infected chickens, served as the negative control during the experiment.

3. Biologycal assay on Issa Brown chickens, 11 days old. A comparative study of the subunit vaccine and of the TB Mukteswar vaccine (mesogenic strain) on Issa Brown chickens, 11 days - old, showed that in the group of 50 chickens vaccinated with the subunit vaccine and artificially infected on day 42 of the experiment, survival amounted to 100%; whereas in the other group of 50 experimental chickens vaccinated with the TB Mukteswar vaccine and artificially infected (in the above described way) 42 days after the vaccination, survival was 80% (40 chickens survived the artificial infection).

A comparison of the titres of HI antibodies agains the Newcastle Disease virus in the sera of the two groups of vaccinated and not infected experimental chickens displayed no sigificant differences during a period of 16 weeks.

The geometric mean titre of the HI antibodies (Gmt log 2/25 ml) in the sera of the Issa Broun chickens, vaccinated and revaccinated with the subunit vaccine was 3,1 six months after the vaccination, and 2,6 one year later.

DISCUSSION

The obtained results confirmed that the subunit vaccine against atypical fowl plague has marked immunogenic properties and provides solid protection to the vaccinated chickens from an infection with the virulent strain of the Newscatle Disease virus. In addition, it can be noticed that very low viral protein concentrations of 0,034 mg/ml in the vaccine, with a hemagglutinating activity of 128 HJ/0,1 ml enabled survival for the vaccinated Hybro and Issa Brown experimental chickens from the artificial infection with 200 000 and 400 000 EID50 of the virulent strain of the Newcastle Disease virus - Hertz 33 (from 74% to 100%).

The immunogenicproperties of glycoprotein antigens (hemmaglutinin-neuraminidase and fusion proteins) isolated from outer envelopes of the Newcastle Disease virus, La Sota strain were confirmed in the biological assays on the experimental chickens as described by Cajavec (1977.) and Cvetnić et al. (1978.).

The results of the studies carried out in this field so far, (Nemarnik, 1978); Čajaved et al., 1982), have shown that experimental subunit vaccines, with higher concentrations of viral proteins than inour vaccine, have a weaker immunogenic effect.

Our experimental subunit vaccine against Newcastle Disease virus elicits a satisfactory humoral immune response in the organisms of immunized chickens and protects vaccinated animals from artificial infection with 200 000 and 400 000 EID50 of NDV, Hertz 33 strain (i/m per animal).

On the basis of the results obtained it can be concluded that:

1. The subunit vaccine with a minimal concentration of viral proteins of 0,034 mg/ml and hemagglutinating activity of 128 HJ/per one dose, induced in all the vaccinated chickens a synthesis of virus - neutralizing HI antibodies with a satisfactory titre.

2. Survival after the artificial infection in Issa Brown experimental chikens, vaccinated with the subunit vaccine and artificially infected on day 28 and 40 of the experiment with 200 000 EID50 of the virulent strain of the Newcastle Disease virus - Hertz 33, was 95,4%.

3. Survival after the artifical infection in Hybro experimental chickens, vaccinated with the subunit vaccine and artificially infected on day 23 of the experiment with 200 000 and 400 000 EID50 of the virulent strain of the Newcastle Disease virus - Hertz 33, was 94% and 74%.

4. Survival among Issa Brown experimental chickens, 11 - days old, immunized with the subunit vaccine and artificially infected on day 40 of the experiment with 200 000 EID 50 of the virulent strain of the Newcastle Disease virus - Hertz 33 (i/m per animal), was 100%.

5. In experimental chickens of the same breed and age, immunized with the TB Mukteswar vaccine and artificially infected on day 40 after vaccination with 200 000 EID 50 of the virulent strain of the Newcastle Disease virus - Hertz 33 (i/m per animal), the survival after artificial infection was 80%.

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ISPITIVANJE IMUNOGENOSTI EKSPERIMENTALNE SUBJEDINIČNE VAKCINE PROTIV VIRUSA NEWCASTLE BOLESTI

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SADRŽAJ

Ispitivanje imunogenosti eksperimentalne subjedinične vakcine protiv virusa Newcastle bolesti živine izvršili smo biološkim ogledom na 180 pilića provinijencije Issa Brown (80 pilića starih 21 dan i 100 pilića starih 11 dana) i 50 pilića provinijencije Hybro starih 21 dan. Eksperimentalnu subjediničnu vakciju pripremili smo od prečišćenih glikoproteinskih subjedinica izolovanih iz spoljašnjeg omotača virusa Newcastle bolesti, soj La sota, hemaglutinacionog titra od 128 HJ/0,1 ml. Antigeni su adsorbovani na adjuvans Al(OH)3, rastvoren u 100 mmol/1 PBS-u. Dve grupe od po 22 i 25 eksperimentalna pileta Issa Brown, imunizovane su sa 0,2 ml subjedinične vakcine i/m po jedinki i revakcionisane 21-og dana ogleda. Grupa od 22 eksperimentalnih piliča veštački je inficirana 28-og dana od vakcinacije sa 200 00 EID 50 virulentnog soja virusa Newcastle bolesti, Hertz33 (i/m po piletu). Procenat preživljavanja veštačke infekcije iznosio je 95,4%. Srednji geometrijski titri HI antitela protiv virusa Newcastle bolesti (Gmt log 2/25 ml) u serumima prve grupe vakcinisanih pilića iznosili su 7-og dana od vakcinacije 2,80, 21-og dana 2,70, 28-og dana 4,0, a u serumima druge grupe pilića iznosili su 7-og dana od vakcinacije 2,87, 21-og dana 2,78 i 28-og dana 4,17. Dve grupe pilića Issa Brown od po 18 i 15 životinja poslužile su kao negativna i pozitivna kontrola u toku ogleda Drugi deo ispitivanja izvršen je u biološkom ogledu na pilićima Hybro starim 21. dan.. Dve grupe od po 16 i 14 eksperimentalnih pilića Hybro, imunizovane na opisan način, veštački su inficirane 21-og dana od vakcinacije sa 200 000 i 400 000 EID 50 soja Hertz 33 (i/m po piletu). Preživljavanje veštačke infekcije je iznosilo 94% kod prve grupe, a 74% kod druge grupe pilića. Druge dve grupe od po 10 nevakcinisanih pilića Hybro poslužile su kao pozitivna i negativna kontrola u ogledu. Uporedna ispitivanja eksperimentalne subjedinačne vakcine i TB-Mukteswar vakcine izvršena su u biološkom ogledu na dve grupe od po 50 pilića Issa Brown starih 11 dana. Preživljavanje veštačke infekcije sa 200 000 EID 50 virusa New Castle, Hertz 33, kod pilića vakcinisanih sa subjediničnom vakcinom iznosilo je 100%, a kod pilića vakcinisanih sa vakcinom TB Mukteswar 80%.