J. Egypt. Vet. Med. Ass. 51, No. 1&2, 387-400 (1991)

STUDIES ON FACTORS INFLUENCING *IMMUNE RESPONSE IN CHICKENS*

III. Effect of Ultracorn on chicken immune response to Newcastle disease virus vaccine.

R. SOLIMAN, I. REDA, S.A.H. YOUSSEF AND M. REFAI

Depts. of Microbiology and Pharmacology, Faculty of Veterinary Med., Cairo Univ. Giza, Egypt.

SUMMARY: The immunomodulating effect of ultracorn (a complete lysate of corynebacterium cutis) on chicken immune response to Newcastle Disease Virus (NDV) vaccine was evaluated.

The vaccination with NDV-vaccine (Lasota strain) was conducted at 14 days old. Ultracorn therapy, however, was given either simultaneously with the vaccine or at 3 days before or 3 day's after the NDV-vaccination in three seperate chicken groups. Ultracorn was given by I/M injection in a dose of 40 µl/chick. A positve NDV-vaccine control group without ultracorn treatment was involved.

The adminstration of ultracorn induced marked immunopotentiating effect of the immune response developed against NDV-vaccine in chickens, especially when given simultaneously with vaccine. The antibody titres (expressed as Geometric Mean Titres-GMT-) reached to 19.7, 39.4 and 27.9 when measured two weeks post vaccination in chicken groups treated with ultracorn at 3 days before, simultaneously and 3 days after vaccination with

This study has been financed by the NARP project No. CAV MI 345 AN03 as a part of that project.

(Received. 2.12.1990)

NDV-vaccine respectively, compared to a GMT titres of 13.9 in the vaccine control group measured at the same time intervals.

Also, a marked immunopotentiation of the in-vitro phagocytic activity of macrophages obtained from ultracorn treated chickens was observed.

The resistance of chickens vaccinated with NDV vaccine and treated with ultracorn to challenge with virulent NDV-virus strain was improved and the rate of protection reached to 90% compared with 70% protection in chicken vaccinated with NDV vaccine without ultracorn treatment.

INTRODUCTION

The current aspect in immunological literature is concerned mainly with the use of non-specific immunostimulants or adjuvants to rise animal resistance to infection.

Adjuvants (L. adjuvare; to help) is a term applied for substances which when introduced into the animal body with an antigen, it potentiate the immune response developed against the antigen (Fudenberg et al., 1978).

Several types of adjuvants are reported in literature. These include Freund's complete adjuvant (Freund, 1951), insoluble particles of aluminum phosphate on which the antigen particles have been adsorbed(Ramon, 1926 and Glemny et al., 1926), *Corynebacterium parvum* (Scott, 1979) and many other adjuvants (Vanselow, 1987).

Corynebacterium parvum, a gram positive bacteria, is a systemic adjuvant used ror immunotherapy as a heat killed and formaldhyde-treated suspensions given orally or injected parenterally either directly into the lesion or at distant sites (Scott, 1979).

The immunopotentiating effects of *C. paravum* have been demonstrated by several authors (Gelenser et al., Geresi et al., 1980) Malhorta, 1984 and Givrged and Comprean, 1985).

The aim of the present work was to elucidate the immunomodulating effect of the commercially available *Corynebacterium cutis* prepartion (ultracorn) on the immune response developed against the NDV vaccine in chickens.

MATERIAL AND METHODS

Materials:

1. **Ultracorn*:** A complete lysate bacterial extract which is produced by ultrasound treatment of a *Corynebacterium cutis* strain. The drug was obtained from Virbac Co. France.

2. **Chickens:** One hundred and fifty, one day's old male L.S.L. chickens were obtained from the General Poultry Company. They were reared and fed on a balanced commercial poultry ration and were kept under standard hygienic conditons.

3. **Newcastle Disease Virus (NDV) strains:**

a. Vaccinal strain:

Lasota NDV strain (Lot. No. 90286 B) produce by intervet International B.V. - Boxmeer, Holland.

b. Challenge strain:

Velogenic viscerotropic NDV strain was kindly supplied by Abassia Serum Institute, Department of NDV vaccine production. It contained 10^9 virion/ml EID_{50}.

4. Other materials: Foetal calf serum, RPMI-1640 tissue culture medium, Ficoll-isopaque (Sp. gr.1.075), PBS. pH. 7.2, preservative free heparin powder, and *Candida albicans* strain serotype A (ATCC 28367).

Experimental design:

Chickens were divided into 5 groups (I to V) each contained 30 chickens. All groups, except groupt I, were vaccinated at day 15 with NDV vaccine via eye drop instillation. Group 1 served as non vaccinated negative control.

Chickens in group III, IV and V were injected 1/M with ultracorn preparation (40 ul/chick). Chickens in group II, however, served as NDV-vaccine control.

Ultracorn was given to chickens in group III at 3 days before vaccination. In group IV it was given simultaneously with the NDV vaccine, while chickens in group V received ultracorn 3 days post vaccination.

From chickens in all the five groups, blood samples were collected before vaccination and 1,2 and 3 weeks post vaccination. Serum was separated and used for evaluation of the immune response developed against the NDV-vaccine. The antibody titres were measured using haemagglutination inhibition test. Also the effect of ultracorn treatment on chicken resistance to challenge with virulent NDV strain was tested. Using the phagocytosis assay the effect of ultracorn therapy on the phagocytic activity of chicken macrophages was determined.

Methods:

1. Haemagglutination inhibition test: It was carried out according to Takatsy, 1956
The antibody titres measured were expressed as Geometric Mean Titres (GMT) according to Brugh, (1977).

2. Challenge Test: Ten chickens from each group were challenged at the end of the 4th week post-vaccination with the virulent NDV strain. The titres of the challenged virus was adjusted to 10^6 virion/ml PBS, then each chick received 1 ml by I/M route. Challenged birds were kept under observation for 21 days. The percentage of protection was calculated and recorded.

3. Phagocytosis assay: was performed according to Richardson and Smith, (1981).

RESULTS

a. Effect of ultracorn on the antibody production in chicken.

As shown in Table 1 and Figure 1 treatment of chickens with ultracorn improved markedly the immune response developed against the Newcastle disease virus vaccine. The most potent immunopotenting effect of ultracorn was observed when the drug was given simultaneously with the vaccine (Group IV), where the antibody titres (expressed as GMT) reached to 7.0, 39.4 and 7 as measured 1,2 and 3 weeks post vaccination respectively, compared to GMT of 4.0, 13.9 and 5.7 in the vaccine control group measured after the same time intervals.

Also it was found that the adminstration of ultracorn three days post vaccination had a significant immunopotentiating effect where the antibody titres reached to 4.9, 27.9 and 8.0 (GMT) measured 1,2 and 3 week

post vaccination respectively, compared to GMT of 4.0, 13.9 and 5.7 in the vaccine control group.

The least immunopotentiating effect of ultracorn was observed when the drug was given perior to NDV vaccination (group III).

Table (1): Effect of Ultracorn therapy on antibody production developed against Newcastle Disease Virus Vaccine in chickens.

Group	Treatmet	Titres of anti-NDV antibodies expressed as GMT (Geometric mean titre) after*:		
		1 week	2 weeks	3 weeks
I	Not treated (Control)	2.6	00.0	4.9
II	Vaccinated (Control)	4.0	13.9	5.7
III	Vaccinated + UC +	4.6	19.7	9.0
IV	Vaccinated + UC ++	7.0	39.4	7.0
V	Vaccinated + UC +++	4.9	27.9	8.0

UC = Ultracorn: The commercial name of C. cutis/preparation supplied by Virbac: Framce
\+ = Administration of UC three days before vaccination.
++ = Administration of UC stimultaneously with vaccination.
+++= Administration of UC three days after vaccination.
(*)= Time intervals post vaccination.

b. Effect of ultracorn therapy on the phagocytic activity of chicken macrophages:

As shown in Table 2 and Fig. 1-A and 1-B, treatment of chickens with ultracorn improved the phagocytic

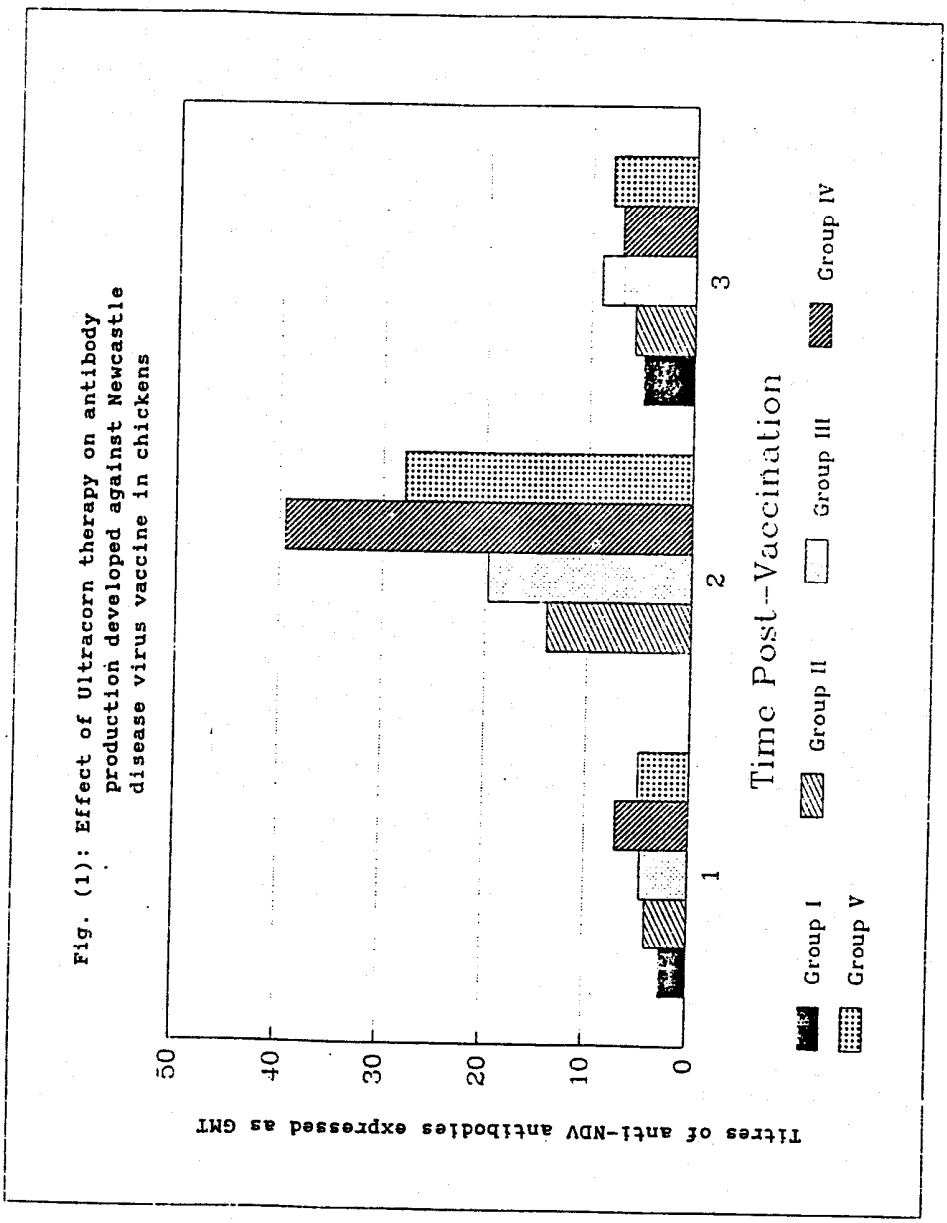


Fig. (1): Effect of Ultracorn therapy on antibody production developed against Newcastle disease virus vaccine in chickens

activity of chicken macrophages where the rate of phagocytosis* reached to 65-68% and the phagocytic index** ranged from 5-7 in the ultracorn treated groups (groups III, IV and V) compared to 1-3 in the ultracorn untreated chickens (groups No. 1 and II).

Table (2): Influence of ultracorn therapy on the phagocytic activity** of chicken macrophages against *Candida ablicans*

Group	Treatment	Rate of phagocytosis	Phagocytic indix
I	No-vaccination No-UC-therapy***	51%	2
III	NDV-vaccination alone	61%	1
III	NDV-vaccination+ UC-3 days before vacc.	68%	5
IV	NDV-vacc. + UC. given simultaneously.	65%	5
V	NDV-vacc. + UC given 3 days after vaccination.	68%	7

* Phagocytic percent:

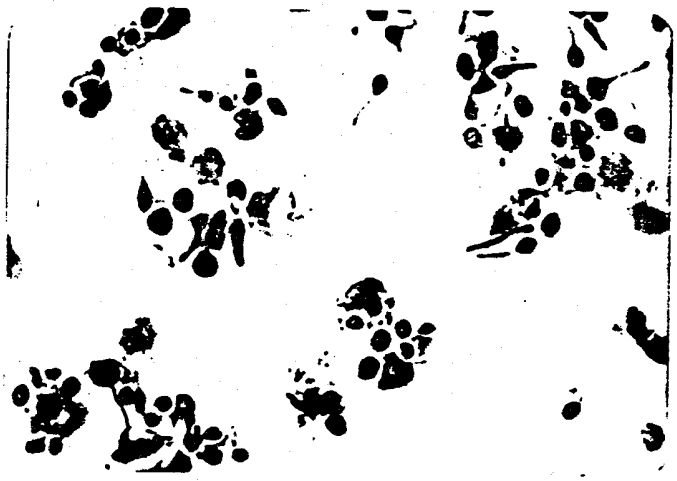
$$\frac{\text{No. of cells phagocytosing C. albicans}}{\text{Total No. of counted cells}} \times 100$$

** Phagocytic activity was measured 2 weeks after treatment with ultracorn.

*** Phagocytic index = mean No. of yeast cells/phagocyte.

$$= \frac{\text{No of yeast cells counted in 100 macrophage}}{100}$$

A. Macrophages from untreated control chicken.



B. Macrophages from ultracorn treated chicken.

Figure 1 (A and B): The phagocytic activity of chicken macrophages (72 hours old) against *Candida albicans*

C. Effect of ultracorn therapy on the resistance of NDV-vaccinated chickens to challenge with virulent neurotropic viscerotrpic NDV-strain.

As shown in Table 3, imprvoment of chicken resistance to virulent NDV virus has been observed in the three groups that received NDV vaccine and the ultracorn preparation (groups III, IV and V) where the protection rates reached to 90% compared to 70% in the NDV-vaccinated control group (group II).

Table 3: Results of challenge test for evalution of the effect of ultracorn therapy on the resistance of NDV-vaccinated chickens to virulent Newcastle disease virus strain.

Chicken gr.	Treatment	Died/Total	Survival rate
I	No. NDV-vaccine No. ultracorn	6/10	40
II	NDV-vaccine only.	3/10	70
III	NDV + UC*	1/10	90
IV	NDV + UC**	1/10	90
V	NDV + UC***	1/10	90

* : Ultracorn was given 3 days before NDV vaccine.

** : Ultracorn was given simultaneously with the NDV vaccine.

*** : Ultracorn was given 3 days after NDV-vaccine.

DISCUSSION

In this study, it was quite clear that treatment of chickens with ultracorn preparation supplied by Virbac as a complete lysate of *Corynebacterium* cutis had a powerful effect on the immune response against NDV-virus.

First of all, ultracorn imporved markedly the antibody titre when given simultaneously with the vaccine where it reached 7.0, 39.4 and 7.0 compared with 4.0, 13.9 and 5.7 in the control group when measured 1,2. and 3 weeks post-vaccination respectively. This result coincides with that obtained by White (1967). Padany et al. (1980) reported that inactivated corynebacterium suspension given as immunomodulator to mice immunized with inactivated *E.Rhusiopathiae* vaccine had increased the vaccine potancy 1.35-2.15 times when given simultaneously with such vaccine. This agrees with our result. However, they reported that such treatment had no effect on the development of immunity, or even suppressed it, when given before or after the vaccine. This result disagrees with our result in which the antibody titres were also elevated when ultracorn was given 3 days before vaccination where it reached 4.6, 19.7 and 9.0 or 3 days after vaccination with antibody GMT titres of 4.90, 27.9 and 8.0 when measured 1,2 and 3 weeks post-vaccination respectively.

Furthermore, the phagocytic activity of macrophages was higher in ultracorn-treated groups in which the phagocytic rate reached to 65-68% compared with 51-61% in ultracorn untreated chickens when measured 2 weeks post vaccination. Also the extent of phagocytosis increased markedly where macrophages from ultracorn treated chickens ingested large number of yeast cells (5-7/cell) compared to those from ultracorn untreated chickens (1-3 yeast/cell). This may coincide with the results obtained by Malhorta et al., (1984) who observed an increase in phagocytic activity of monocytes of 6 healthy Jersey calves 2 weeks after being subcutaneously inoculated with 200 mg of Killed. *C. parvum.*

Moreover, ultracorn was found to enhance the degree of protection against virulent NDV in all ultracorn treated groups in which the survival rate reached 90% compared to 80% in vaccinated control group and 50% in the non-vaccinated group. This agrees with

the results obtained by Gelencser et al., (1980) who observed active protection in mice immunized with tetanus toxoid administrated simultaneously with vaccine prepared from 6 strains of conrynebacterium and that obtained by Corrier and Wagner (1984) who observed complete protection of mice treated intraperitoneally with 10 mg/kg *C. parvum* and challenged intraperitoneally 3 days later with 10^6 *Babesia rodhain* that caused death of all untreated control mice.

REFERENCES

1. Aiyedun, B.A. (1986 a): Adjuvant and the reticulothelial system: the stimulatory effect of M. tuberculosis and Corynebacterium parvum in chicken. Bull. Anim. Hlth. and Prod. Africa, 34 (4): 246-252.

2. Aiyedun, B.A. (1986 b): Comparison of the adjuvant activity of Mycobacterium tuberculosis and Corynebacterium parvum. Bull. Anim. Hlth. and Prod. Africa, 34 (4): 242-244.

3. Brugh. M. Jr. (1977): A simple method for recording and analyzing serological data. Avian Dis. 22: 362-365.

4. Corrier, D.E. and Wagner, G.G. (1984): The protection effect of pretreatment with killed Corynebacterium parvum against acute babesiasis in calves.

5. Freund, J. (1951): The effect of paraffin oil mycobacteria on antibody formation and sensitization. Am. J. Clin. Pathol. 21, 645-656.

6 . Gelencser, F., Rethy, L.; Bacokai, L.; Geresi, M; Pogany, I. Padanyi, M. and Rethy, L.A.Jr. (1980): The effect of anaerobic corynebacterial immunostimulants on the primary immune response to tetanus toxoid and perfringns epsilon toxoid (short communication). Acta Vet. Sci. Hungaricae, 28 (3): 285-287.

7 . Geresi, M.; Rethy, L.; Ecsy, R.; Gelencser, F., Padani, M.; Pogany, I.; Bacskai, L.; Pethy, L.A. Jr. (1980): Antitumor effect of different corynebacterial immunostimulants in non isogenic mouse tumor system (short communication). Acta. vet. Acad. Sci. Hungaricae, 28 (3): 281-283.

8 . Givergea, R. and Coppreаn. D. (1985): Reaction of the thymus, and bursa of Fabricius in chicken inoculated with Corynebacterium parvum. Acta Vet. Acad. Sci. Hungaricae, 33 (4) 163-168.

9 . Malhorta, D.V.; Gahlot, A.K.; Dhas, S. and Gautam, (1984): The effect of non specific immuostimulations with corynebacterium parvum on phagocytic activity of monocytes in bovine calves. Ind. J. Vet. Med., 4 (2): 77-97.

10. Padany, M.; Reth, L.; Kulcsar, A.; Geresi, M., Gelencser, F.; Pagany, L., Bacskai, L. and Rethy, L.A.Jr. (1980): the development of primary antibacterial immune protection against Erysipelothrix rhusiopathiae and the effect of corynebacterial immunostimulants (short communication). Acta Vet. Acad. Sci. Hungaricae 28 (3): 273-275.

11. Ramon, G. (1926): L'anatoxine diphterique et les anatoxines en general etude experimentale (Revue): general etude experimentale (Revue): J. Med. Franc. 15, 381.

12. Richardson, M.D. and Smith, H. (1981): Resistance of virulent and attenuated strains of Candida albicans to intracellular killing by human and mouse phagocytes. J. Infect. Dis. 114: 557-565.

13. Scott, M.T. (1979): Anatysis of the principles underlying chemoimmunotherapy of mouse tumors 1: Treatment with cyclophosphamide followed by Corynebacterium parvum. Cancer Immunol. Immunsther. 6: 107-177.

14. Takatsy, G.Y. (1956): The use of spiral loops in serological and virological micromethods. Acta Microbiologica Hung. 3, 197.

15. White, R.G. Henderson, D.C., Eslams, M.B., and N. Elsen., K.H. (1975): Focabration of protein antigen in chicken spleen. Effect of various manipulative procedures on morphogensis of the general center. Immunol. 28, 1.

16. Fudenberg, H.H., Stites, D.P. Caldivell, J.L., and J.V. Wells (1978): Basic and chemical immunology 2ed Ed. Lange Medical Publications, Los Altos, California, U.S.A.

12. Richardson, M.D. and Smith, H. (1981): Resistance of virulent and attenuated strains of Candida albicans to intracellular killing by human and mouse phagocytes. J. Infect. Dis. 114: 557-565.

13. Scott, M.T. (1979): Anatysis of the principles underlying chemoimmunotherapy of mouse tumors 1: Treatment with cyclophosphamide followed by Corynebacterium parvum. Cancer Immunol. Immunsther. 6: 107-177.

14. Takatsy, G.Y. (1956): The use of spiral loops in serological and virological micromethods. Acta Microbiologica Hung. 3, 197.

15. White, R.G. Henderson, D.C., Eslams, M.B., and N. Elsen., K.H. (1975): Focabration of protein antigen in chicken spleen. Effect of various manipulative procedures on morphogensis of the general center. Immunol. 28, 1.

16. Fudenberg, H.H., Stites, D.P. Caldivell, J.L., and J.V. Wells (1978): Basic and chemical immunology 2ed Ed. Lange Medical Publications, Los Altos, California, U.S.A.