

EFFECT OF GAMMA IRRADIATION ON THE PROPAGATION OF SOME POULTRY VIRUSES IN EMBRYONATED CHICKEN EGGS AS (NEWCASTLE, INFECTIOUS BURSAL DISEASE AND INFECTIOUS LARYNGOTRACHEITIS VIRUSES)

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ABSTRACT

A large number of embryonated chicken eggs (ECEs) were irradiated at low doses (100, 150 and 200 rads). Newcastle disease virus (NDV) was also irradiated with the same doses and titrated in the irradiated ECEs using Reed and Muench (1938). Infectious bursal disease (IBDV) and infectious laryngotracheitis (ILT) viruses were also irradiated at the level of (25, 50, 75, 100 and 200 rads) in the three form (lyophilized, reconstituted and fluid vaccine before lyophilization). The results showed a stimulatory effect of gamma irradiation on the viruses used in this study represented by increased titre.

INTRODUCTION

The use of the manufactured radiation was gained an special importance during the last 20 years. The use of the ionizing irradiation has been explored in the production of non-infective antigens for vaccines as well as for diagnostic and therapeutic purpose. The major practical application of ionizing irradiation is depend on the constraints of cost and user safety, beside to the sterilization of materials that would be damaged by heat. In more highly developed countries, exposure from medical sources may be equal or exceed natural background radiation but in un-developed countries the relative contribution of the medical irradiation may be only about 5% of the total exposure (Mettler et al., 1985). The exposure of living tissue to gamma rays produces ionization inside their cells. This alteration causes biochemical and physiological changes depending upon the dose level and the stage at which the tissues were irradiated. Fowl eggs are the most convenient biological system for tracing the ionizing radiation from early stage to the productive stage. The aim of this study is to provide a base line information concerning the haz-

ard and benefit effects of applying low doses of the gamma rays on the propagation of some poultry viruses in the embryonating chicken eggs (ECEs).

MATERIAL AND METHODS

Embryonated chicken eggs (ECEs) :

9-11 day old ECEs were obtained from United Company for Poultry Production.

Viruses :

1. Newcastle disease virus (NDV) :

LaSota strain was supplied by the Central Veterinary Laboratory, Weybridge, England and was used in the preparation of ND vaccine. Its titre was 10^{10} EID₅₀/ml.

2. Infectious bursal disease virus (IBDV) :

IBDV vaccine (bursa vac strain) locally prepared in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. Its titre was $10^{7.2}$ EID₅₀/ml.

3. Infectious laryngotracheitis virus (ILT) :

Locally prepared ILT vaccine in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. Its titre was $10^{5.5}$ EID₅₀/ml.

Viruses titration in embryonated chicken eggs :

It was carried out according to Anon (1971). The EID₅₀ was calculated by the method of Reed and Muench (1938).

Irradiation source :

The source of irradiation was Co⁶⁰ gamma cell at the Egyptian Atomic Energy Authority at Cairo. The doses were 25, 50, 75, 100, 150 and 200 rads. The rad : minimal unit of adsorbed dose.

Experimental Design :

Two experiments were carried out in this study :

1st experiment : In which ECEs and NDV fluid were irradiated at the range of 100, 150 and 200 rads. Inoculation was done as the following diagram.

NDV :

1. Normal virus. Its titre was 10^{10} EID₅₀/ml.

2. 100 rads irradiated virus.

3. 150 rads irradiated virus.

1, 2 and 3 viruses were inoculated each alone once in irradiated ECEs (100 rads), in irradiated ECEs (150 rads) and in irradiated ECEs (200 rads).

2nd experiment :

In which the infectious laryngotracheitis (ILT) and infectious bursal disease (IBD) viruses were irradiated at the range of (25, 50, 75, 100 and 200 rads) in 3 forms lyophilized, reconstituted (after lyophilization) and as a fluid (before lyophilization) and ultraled in normal ECEs.

RESULTS AND DISCUSSION

Electromagnetic radiation is divided into non-ionizing and ionizing radiation according to the energy required to eject electrons from the molecules (Sanders, 1986). The ionizing radiations that behave more like waves of energy include X-rays and gamma rays. Radiation exposure comes from many sources and may be directly ionizing or indirectly ionizing. Directly ionizing radiation carries an electric charge that directly interacts by electrostatic attraction or repulsion with atoms in tissue or medium exposed. Indirectly ionizing radiation is not electrically charged but results in production of charged particles by which its energy is absorbed. Exposure to ionizing radiation leads to the formation of free radicals (from non-aqueous compounds of the proloplasm and water) which combine with DNA, RNA and other important cellular constituents to damage them. These free radicals are chiefly oxidants. The more drastic effects of the gamma rays on the cell structures results in the liberation of the hydrolytic enzymes of lysosomes (Tarasenko, 1968) and increase in cell membrane permeability (Stem, 1960). In biological system, the effect of ionizing radiation differs in qualitative manner when radiation dose is changed, small doses act by indirect action and produce mainly oxidation whereas large doses act by the two mechanisms (Hollaender, 1954).

This present study aimed to reveal the effect of the small irradiation doses on the viruses propagation in ECEs estimated by virus ultraled. The results of the 1st experiment showed that the highest increase in NDV titre is obtained in case of inoculation of NDV irradiated at (100 and 150 rads) in normal ECEs reached 10^{14} and 10^{13} EID₅₀/ml, respectively, followed by that inoculated (by normal and irradiated NDV at 100 and 150 rads) into irradiated ECEs at 200 rads. This increases in the virus titre may be explained on the base of exposing the fertile eggs to low doses of ionizing radiation may activate cell division. Magda (1995) and Todorovic et al. (1986) added that low doses of gamma irradiation possible influence the nuclei or change the chromo-

some structure or special genes in the chromosomes.

It is important to mention that the appearance of haemagglutination assay which used as a tool to evaluate the alterations caused by radiation on the molecular arrangement of viral proteins was changed. The same results were obtained by **Silva et al. (1995)** who noticed an alterations of the virus haemagglutinating activity observed only when radiation levels were higher.

The results of the 2nd experiment revealed that the sensitivity of IBDV to irradiation is increased by the increasing of the irradiation dose (Fig. 1) as well as the sensitivity of lyophilized vaccine is more than the fluid one. Similar results were obtained by **Mahnel et al. (1980)** who stated that the resistance to radiation being higher with a higher protein content and with freeze drying state. In concern to ILTV, the inoculation of irradiated virus at (50 rads) reconstituted and lyophilized vaccine gave the highest titre reached $10^{7.25}$ EID₅₀/ml while the control was $10^{5.5}$ /ml EID₅₀. Followed by irradiated lyophilized virus at (75 rads) reach also $10^{7.25}$ EID₅₀/ml. In general, there is a marked increase in different viruses titres regardless of their nucleic acid (RNA or DNA) and these result is in agreement with that obtained by **Mahnel (1980)** who concluded that resistance to irradiation varied widely between viruses and was generally more closely correlated with chemical-physical influence than with type of nucleic acid of the virus. Also type of medium affects the sensitivity.

Fig. 4 : The results of titration of the NDV inoculated in irradiated ECEs.

Radiation dose for NDV	NDV titre after irradiation			
	100 rads ECEs	150 rads ECEs	200 rads ECEs	Normal ECEs
Normal NDV	11	12	14	10
NDV irradiated 100 rads	12	9.5	13	13
NDV irradiated 150 rads	12	10	13	14

The NDV used was fluid vaccine.

The titre calculated as EID₅₀/ml.

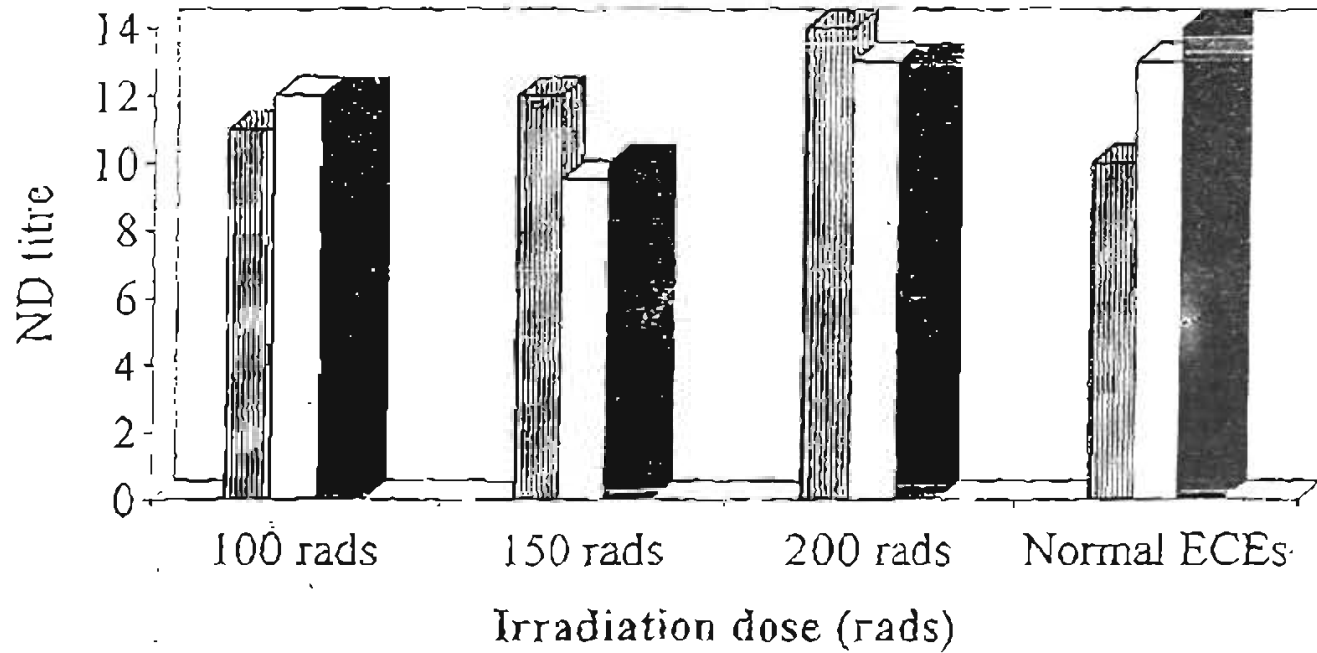
Table 2 : The effect of different doses of gamma irradiation on both infectious bursal disease and infectious laryngotracheitis viruses titre.

Virus form	Different irradiation doses on ECEs									
	25 rads		50 rads		75 rads		100 rads		200 rads	
	ILT	IBDV	ILT	IBDV	ILT	IBDV	ILT	IBDV	ILT	IBDV
Reconstituted	5.80	8.2	7.25	8.9	6.51	9.2	5.9	9.2	6.82	9.0
Fluid vaccine	6.85	7.5	6.90	8.5	6.50	8.3	Not done	9.1	6.40	9.5
Lyophilized	6.70	8.0	7.25	8.5	7.50	8.1	6.7	8.1	6.90	9.5

Titre of ILTV control was $10^{5.5}$ /ml EID₅₀.

Titre of IBDV control was $10^{7.2}$ /ml EID₅₀.

Fig. (1) The results of titration of the NDV inoculated in irradiated ECEs



Normal NDV



NDV irradiated 100 rads



NDV irradiated 150 rads

REFERENCES

- Anon (1971) : Methods of examining poultry biologics and identifying and quantifying avian pathogens. Nat. Acad. Sci. Washington, D.C. Cited by Amer M. M.
- Hollaender, A. (1954) : Radiation biology. McGraw Hill Book Co. Inc., New York.
- Magda, M. M. (1995) : Effect of gamma radiation on Japanese quails. M. Sc. Thesis, Fac. Sci., Ain Shams Univ.
- Mahnel, H.; Brodorott, H. S. Von; Ottis, J. and Von Brodorotti, H. S. (1980) : Sensitivity of viruses to gamma radiation. Inst. Med. Mikrobiol. Fachbereich Tiermedizin Veterinarstr. 13, D 8000 Munchen 22, German Federal.
- Mettler, F. A. Jr. and Moseley, R. D. Jr. (1985) : Medical effects of the ionizing radiation. Grune and Stratton, New York.
- Reed, L. J. and Muench, H. (1938) : A simple method of estimating fifty percent end points. Am. J. Hyg., 27 : 493 - 497.
- Sanders, C. L. (1986) : Toxicological aspects of energy production, pp. 253 - 284 Battelle Press Columbus, OH.
- Stern, C. (1960) : Principles of Human Genetics. 2nd Ed. San. Francisco, Freeman, W.H.
- Silva, Mauro R.; Couceiro, Jose, N. S. S.; Brito, Siederth, N. Cabral and Maudori, C. (1995) : Effects of ultraviolet radiation on structural components of enveloped RNA virus. Universidade Federal, Rio de Janeiro, R.J. Brazil Inst. d. Microbiologia.
- Tarasenko, N. D. (1968) : Effect of gamma rays and fast neutrons on the frequency of chromosome rearrangements and on the survival of potato seedlings. Genetika. 4 : 28 - 32.
- Todorove, B. T.; Chotinski, D. and Cvetanov, I. (1986) : Effect of low doses of gamma irradiation upon hatchability of egg and live weight of the broilers hatched. Page 122 In : Proc. Eur. Nucl. Methods. Agric. XVIIIth Annual Meeting, Institut für Biophysik Universität Hannover 21. B. R. D. (Abst.).

الملخص العربى

تأثير أشعة جاما على نمو بعض فيروسات الدواجن فى أجنة البيض مثل فيروس النيوكاسل وفيروس غدة البرسا وفيروس إلتهاب الحنجرة والقصبية الهوائية المعدى

المشتركون فى البحث

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تم فى هذه الدراسة تسميخ عدد كبير من بيض الدجاج المخصب لجرعات من إشعاع جاما كانت (١٠٠ ، ١٥٠ ، ٢٠٠ راد) (وحدة قياس كمية الأشعة الممتصة) وتم تعريض فيروس النيوكاسل لنفس الجرعات السابقة وتم عمل تبادل بينهما فى الحقن فقد تم حقن البيض المشع (١٠٠ راد) بـ فيروس النيوكاسل المشع ب (١٠٠ ، ١٥٠ راد) والفيروس الغير مشع وهكذا وتم قياس عيارية فيروس النيوكاسل فى كل الحالات. كما تم تعريض كل من فيروس إلتهاب القصبية والحنجرة المعدى والجيمبورو لدرجات متفاوتة من الإشعاع (٢٥ ، ٥٠ ، ٧٥ ، ١٠٠ ، ١٥٠) راد فى صوروتهم السائلة والمجففة بعد إعادة حلها وحقنها فى البيض المخصب عمر ٩-١٠ أيام وغير مشع وحساب عياريتها وقد وجد أن برجه عام زيادة فى عيارية معظم الفيروسات وبعض التغيير فى صورة إختبار منع التلازن.