الجمهورية اللبنانية
مكتب وزير الدولة لشؤون التنمية الإدارية
مركز مشاريع ودراسات القطاع العام

Republic of Lebanon
Office of the Minister of State for Administrative Reform
Center for Public Sector Projects and Studies
(C.P.S.P.S.)

DEVELOPMENT OF A RADIATION-ATTENUATED VACCINE AGAINST INTESTINAL COCCIDIOSIS IN POULTRY
LEB–74–016

LEBANON

PROJECT FINDINGS AND RECOMMENDATIONS

UNITED NATIONS DEVELOPMENT PROGRAMME

INTERNATIONAL ATOMIC ENERGY AGENCY – Vienna, 1976

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Report prepared for
the Government of Lebanon

by

the International Atomic Energy Agency (IAEA)
acting as Executing Agency for
the United Nations Development Programme
and the IAEA Regular Programme of Technical Assistance

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CONTENTS

1. BACKGROUND

1.1. Justification for the project

A tremendous and continuous increase in poultry production has been established in Lebanon during recent years. This resulted in the build-up of one important animal industry of this country, i.e. poultry industry. Lebanon produces a significant amount of high quality protein e.g. meat and eggs. This country is to be regarded as the only centre in the Middle East exporting poultry and other relevant products to the neighbouring countries.

It is appropriate, however, to point out that disease problems have proportionately increased with the great and rapid growth in poultry production. In some countries drug resistant Eimeria strains induced by continued use of coccidiostats are supposed to cause problems in intensive poultry breeding. The latest estimations have indicated that the cost of the disease to Lebanese poultry industry amounts annually to over L.L. 12 million in terms of actual mortality, loss of body weight in broilers and egg production, including the cost of prophylactic and therapeutic medications. The mortality rate attributable directly to coccidiosis is estimated at between 60% and 80% in unmedicated young chick populations.

Serious efforts have already been made to reduce substantially the economic losses in poultry production by introducing immunoprophylaxis as a measure in the control of coccidiosis. Encouraging results have so far been obtained from research work at the Regional Poultry Laboratory, Fanar, on the development of a radiation-attentuated vaccine for prevention of the disease. A potent vaccine against one pathogenic species of poultry coccidia has been prepared on an experimental basis. With this fact at hand it would seem realistic to expect that a potent vaccine against several pathogenic species of poultry coccidia could be developed. A polyvalent vaccine against coccidiosis which might be developed at the Regional Poultry Laboratory, Fanar, would perhaps have some economical implications on poultry production in Lebanon and elsewhere. From a scientific point of view the possible development of a radiation-attenuated vaccine would be a great achievement representing a model of scientific research on the basis of which the research in the field of immuno-parasitology would continue at the Regional Poultry Laboratory with staff already available there.

2. FINDINGS

2.1. Initiation and administrative realisation of the project

The afore mentioned facts (1.1.) constituted the basis upon which the

attenuated vaccine against poultry coccidiosis.

3. PROGRESS ACHIEVED

Work on the project was of a complex nature, requiring detailed planning and accurate synchronization of the critical aspects of the work programme. Top priority was given to the organisation of a working place and to the provision of adequate facilities to ensure satisfactory progress of the work.

3.1. Working space and facilities

Work on the development of a vaccine against coccidiosis in the domestic fowl received full understanding and the constant support of the Fanar authorities. Due to this fact it was possible to rearrange or adapt conveniently the existing space at Fanar to create facilities for carrying out an imposing number of the laboratory procedures in a relatively short time. These included among others (i) diagnosis; (ii) sterilization; (iii) sterile filtration; (iv) tissue culture; (v) histology; (vi) fluorescent microscopy; (vii) electrophoresis; (viii) immunoelectrophoresis; (ix) double diffusion; (x) gel filtration; (xi) column chromatography; (xii) freeze-drying; (xiii) super-speed centrifugation; (xiv) pH-measurements and (xv) microhaematocrit readings.

During the execution of the project laboratory working space was enlarged to about 10 times when compared with the space which was at disposal at the start of the project. A number of rooms needed to place and manipulate experimental animals was increased proportionally with the increasing scope of work.

3.2. Equipment and utensils

The sizeable input in the form of equipment and supplies which were financed by the UNDP and the IAEA regular programme made a substantial contribution to the work undertaken. Consequently, the field of work could be greatly enlarged and the work efficiency noticeably improved in spite of the fact that not all of the requested items had been delivered before the above-mentioned unfortunate break in the work in September 1975.

The main items provided are listed in Appendix. This list of equipment and utensils gives an idea of the potentially large scope of work which was achieved and which could be carried out in the field of immunoparasitology as well as in immunology itself. However, further steps are needed to collect the remaining purchased items which have not been received at Fanar before the submission of this report. A list of a few additional items of the useful laboratory utensils recommended if work on this project is to be continued, is

below.

4.1. A suitable procedure for calibration of a radiation unit (^{60}Co-source) was elaborated. For this purpose a convenient Perspex phantom was constructed. Calibration was an important step in defining geometry of irradiation in the experiments on development of a radiation attenuated vaccine using Eimeria oocysts as antigens [Ref. 6].

4.2. The effect of radiation (10 and 15 krad) on Eimeria tenella oocysts was comparatively studied with normal and irradiated oocysts in chicken kidney cell culture. It was found that cell penetrating capability of the sporozoites was affected at both radiation levels. With progressing time, the radiobiological effect became more marked in terms of growth and development of the different stages in comparison with normal E. tenella. Those stages deriving from normal oocysts represented approximately 57% of the total population, while those from irradiated oocysts amounted to 22% (10 krad) and 21% (15 krad). Normal oocysts developed to the stage of gametogony while irradiated oocysts only reached the stage of mature schizont or liberated merozoites.

Specific antigens were detected in situ in all stages developing from normal and irradiated E. tenella. Good evidence was shown that antigens remained preserved in irradiated oocysts [Ref. 1].

4.3. It was established that chicks given one oral dose of 50,000 irradiated oocysts of E. tenella survived a challenge with 100,000 normal oocysts of the same species given two weeks later. This challenge killed 50% of the control birds. Chickens receiving at a two week interval two oral doses consisting of 50,000 and 100,000 irradiated oocysts survived a challenge with 200,000 normal oocysts - fatal for 40% controls - two weeks after the second dose of irradiated oocysts. All oocysts were irradiated at 10 krad [Ref. 8].

4.4. The results obtained from research on irradiated oocysts of E. tenella, E. brunetti and E. necatrix respectively demonstrated that it was possible to induce strong resistance in chicks (three weeks old), against the subsequent challenge with infective oocysts of the homologous species.

Oocysts of the afore-mentioned Eimeria species were irradiated at 10 krad and a single oral dose of each species was administered to a separate group of birds. All vaccinated chicks and the non-treated control birds were challenged two weeks later. The chicks receiving immunizing dosesof irradiated oocysts survived a massive challenge dose without losses or showing clinical symptoms of the disease, while the challenge dose killed 10% of the control group in the case of E. brunetti and 70% of the controls in the group of E. necatrix and E. tenella respectively [Ref. 11].

fluoresoent antibody staining technique was used for the investigations. It was found that the second generation mature schizont showed the most intensive specific immunofluorescence.

Antibodies specific to E. tenella could be differentiated from those of E. acervulina, E. brunetti, E. maxima or E. necatrix. For this purpose the antibody titration procedure with serial dilution of unconjugated sera and the second generation mature schizont of E. tenella as antigen was used [Ref. 2].

4.9. Immunodiagnosis of E. tenella infection using dried blood was elaborated. Minute amounts of blood were collected from chickens previously infected with E. tenella. Blood from a wing vein was put onto a strip of filter paper on which it was dried. Dried blood samples were then stored 10 days in a refrigerator before being examined. Thereafter blood was eluted from the filter paper and comparatively examined with the corresponding serum sample using immunofluorescence. It was found that immunoglobulins eluted from dried blood retained their complete reactivity producing specific immunofluorescence of the antigen-antibody interactions [Ref. 3].

4.10. Immune response in chickens to E. tenella infection using oral and subcutaneous routes of infection was comparatively studied. The results obtained from these studies indicated that normal sporulated oocysts were not lethal for chickens when innoculated subcataneously with up to 50,000 oocysts. They were found less immunogenic than orally administered oocysts.

The dynamics of antibody response was different comparing the two routes of infection. Orally given oocysts stimulated a dramatic increase in the antibody titre with a subsequent titre decrease after 14 days irrespective whether birds received a second dose of oocysts or not. A third oral dose of oocysts stimulated, however, a slight increase in antibody titre. Two doses of oocysts injected subcutaneously induced only a slight increase in antibody titre this having been dramatically increased after one oral dose of oocysts.

It was demonstrated that antibodies specific to E. tenella were bound to IgM and IgG immunoglobulins [Ref. 5,9.].

4.11 It was demonstrated that infection with E. tenella stimulated production of the specific antibodies in chicks. These were bound mainly to IgG and less to IgM. Antibodies specific to E. tenella and IgG were also present in the caeca of the infected chickens until the 28th post infection day. As they could

Two short scientific visits for the Fanar senior scientists to European institutions engaged in a similar field of research, in order to discuss organisation, management and research programme, are recommended.

Fellowships for two qualified post-graduates assigned for work on the project are recommended when the scheduling of the work will permit.

It seems realistic to expect that much quicker progress in the work on coccidiosis can be done if collaboration of research workers from Fanar, Zemun and other relevant places actively engaged in the problems of poultry coccidiosis be efficiently organised.

6.2. Personal Remarks

The Regional Poultry Laboratory is a place with well developed and equipped departments which include a Department for Vaccine production, Department of Virology, Department of Bacteriology, Department of Parasitology, Post Mortem Section, etc. There are laboratory and animal room facilities for routine work and research in the aforesaid fields. The Fanar authorities support and stimulate equally, practical and research work. Expert's assistance was well received and appreciated. The Fanar staff were cooperative.

If work in the field of immunoparasitology is to continue as successfully as it was carried out under the current project it is my view that the Regional Poultry Laboratory, Fanar, if supported, will soon reach a leading position in immunoparasitology in the entire Middle East area which is seriously faced with problems of many parasitic diseases. It could, in the future, serve as a nucleus for dissemination of knowledge in immunoparasitology in the neighbouring countries.

7. ACKNOWLEDGEMENTS

I wish to express my best thanks to Dr. Haraoui, Director General, Agriculture Research Institute; Dr. A. El-Zein, Director, Regional Poultry Laboratory and Dr. J. Rizk, Former Director, for their support and close co-operation during my work at Fanar. The co-operation of their staff at Fanar and especially of the direct counterparts is gratefully appreciated.

Best thanks are also due to Mr. A.H. Mackenzie, UNDP Resident Representative; Mr. E. Perrin, UNDP Assistant Resident Representative and the UNDP staff in Beirut for their helpful support.

I also wish to thank my colleagues, Dr. M. Movsesijan and Dr. K. Cuperlović for their active co-operation and interest in the work.

APPENDIX

MAJOR ITEMS OF EQUIPMENT PROVIDED

	ITEM	Source of Funds
1.	Pipetting outfits	Regular Programme
2.	Con-torque power unit with accessories	Regular Programme
3.	LKB precision column with cooling jacket	Regular Programme
4.	UGI-apparatus, macro-, semi-macro- and micro-procedures; power supply unit; gel-diffusion equipment	UNDP
5.	LKB Ultro-rac fraction collector, with accessories	UNDP
6.	Leica MDA camera with accessories	UNDP
7.	M-rotor, HL-4 roter, omni carrier and GLC-2	UNDP
8.	Large sliding microtome	UNDP
9.	Sterilizer, model TV 40 SL	UNDP
10.	LKB precision pH-meter with accessories	UNDP
11.	LKB LL-UF-2 Concentrator, instant dialyzer and accessories	UNDP
12.	Mod. EF03 Centrifugal freeze-dryer with accessories	Regular Programme
13.	Digital precision balance	Regular Programme
14.	RC-5 Centrifuge with accessories	Regular Programme
15.	Liquid nitrogen containers, controller, etc.	Regular Programme
16.	LKB 3 Precision columns with cooling jackets	UNDP

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