العنوان:	دراسة مناعية و سيرولوجية على ميكروب لبوريليا انسرينا في الدجاج
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مؤلفين آخرين:	فريد، أحمد فريد(مشرف)
التاريخ الميلادي:	1981
موقع:	الزقازيق
الصفحات:	1 - 110
رقم MD:	544047
نوع المحتوى:	رسائل جامعية
اللغة:	Arabic
الدرجة العلمية:	رسالة دكتوراه
الجامعة:	جامعة الزقازيق
الكلية:	كلية الطب البيطري
الدولة:	مصر
قواعد المعلومات:	Dissertations
مواضيع:	الطب البيطري، أمراض الدجاج، المناعة، الميكروبيولوجيا
رابط:	http://search.mandumah.com/Record/544047

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DEDICATION

THIS THESIS IS DEDICATED TO

MY DEAR PARENTS

AND MY ANGEL

WHO OFFERED A LOT FOR ME

ACKNOWLEDGEMENT

I would like to take this opportunity to express my cordial gratitude and deepest thanks to Prof. Dr. Ahmed Farid, Ex-Chairman and recently Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University for his stimulating supervision, constructive criticism and constant advice. Indeed the completion of this work was impossible without the extraordinary fruitful efforts willingly given by him.

I am also greatly indebted to Dr. Nour El-Din Amin, Assistant Professor of Microbiology, Faculty of Veterinary Medicine, Zagazig University who associated in the supervision, for his guidance, valuable help and encouragement willingly given to me during this work.

My thanks and gratitude also extend to Prof. Dr. IV. Djankov and Prof. Dr. A. Packchanian for sending of some valuable research papers as soon as they requested.

Finally I have the pleasure to extend my thanks to all my colleagues in the department for the help and encouragement provided to me by them.

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INTRODUCTION

Poultry industry plays an important role for fullfilling the excessive demands of the increased population from the animal protein. Special attention was payed towards it in order to save such essential source of meat and eggs necessary for the human consumption.

Although an unnegligible part of this national source is still in the hands of the villagers, where there is a notable shortage of the veterinary service and hygeinic measures giving a yet chance for the outbreak of some fatal diseases, the efforts for giving the advice on poultry keeping and hygeine to such persons are fruitless.

Avian borreliosis is a disease caused by Borrelia anserina of genus Borrelia, Family Treponemataceae of the order Spirochaetales, which was firstly observed by Sakharoff (1891) in the blood of sick geese in Russia. Further investigations were performed for studying the organism to clear out its descriptions and pecularities.

Concerning the antigenicity of this organism, which is still a point of argument, the study of this problem as may be seen from the literature is far

from being concluded. Many of the investigators contradict one another and some have not been confirmed by further studies or attached this point in different ways.

The prevention of avian borreliosis depends upon the elemination of the transmitting vectors, a policy which is very difficult to be adopted in Arab Republic of Egypt as well as the adoption of certain measures of immunization and treatment. But the prevalence of a number of antigenically variant strains of Borrelia anserina in a locality supports the more accurate direction for preparation of a polyvalent vaccine incorporating the highly immungenic strains and its use in massive vaccination of the birds against this disease instead of the monovalent vaccine which proved to be of limited protective ability.

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AIM OF THE STUDY

The present work has been planned to cover the following points:

1. Isolation of different Borrelia anserina strains from various districts distributed allover Sharkia Governorate.
2. Determination of the antigenic structure of these isolates by means of: Agglutination test, Immobilization-lysis test and Agar gel precipitation test.
3. Meticulous comparison of the abovestated serological reactions in order to select the highly antigenic strains to be used for the preparation of the polyvalent vaccine.
4. Trials to use such polyvalent vaccine for immunization of fowls and testing its immunizing power by a challenge test after its inoculation with both homologous and heterologous strains and their protection by vaccination with such vaccine.
5. Experiments to explore the more potent curative drugs as a method for treatment and control of this fatal disease.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

The available literature entaling this study can be classified under the following categories:

I- Isolation of Borrelia anserina:

Sakharoff (1891) was apparently the first who observed Borrelia anserina, the cause of avian borreliosis, in the blood of the sick geese in Caucasus (Russia). He mentioned that Spirochaeta gallinarum could infect chickens, ducks, geese, rabbit and sparrows, but Spirochaeta anserina was only restricted for chickens.

Marchoux and Salimbeni (1903) isolated the organism from fowls in Rio-deJaneiro and stated that the Brazilian organism was different from the Caucasian variety, suggesting that it may be a virus.

Borrel and Burnet (1906) were the first who made a trial for the isolation of the organism by cultivating it on citrated blood after the addition of sterile serum. They stated that the organisms can not grow further than the second generation.

Balfour (1907) isolated the organism from fowls infected with the disease at Kartoum. He mentioned that the spirochaetes isolated from infected fowls in Sudan may be different from the Brazilian virus.

Hindle (1911) recovered his strain from fowls infected with the disease in citrated blood kept in sealed tubes and stated that spirochaetes could be kept alive for 3 months in infected citrated blood.

Noguchi (1912) isolated Borrelia anserina strain by cultivation is a deep column of ascitic fluid to which a fresh tissue in the form of a fresh rabbit kidney or a piece of chicken pectoral muscle is added.

Neiva (1914) stored his strain in defibrinated blood kept in ice chest at 0°C and he mentioned that borreliae could stand alive and virulent for forty-nine days.

Galloways (1925) kept borreliae by its cultivation on media consisting of egg albumin and rabbit or horse serum diluted with sterile saline put in slope tubes covered with a layer of sterile paraffin.

Anderson (1928) secured his strain in citrated blood periodically passage in fowls. He stated that borreliae lost their virulence which is revived by its passage through ticks.

Kligler et al. (1938) obtained borreliae by its cultivation on Tyrode's serum which consisted of one part of rabbit serum and two parts of Tyrode's solution.

Sreenivasan and Sank (1943) isolated borreliae in citrated blood kept in the refrigerator at 4°C. They added that the virulence of the organisms in blood samples could be kept by this way for at least 97 days.

El-Dardiri (1945) in his study on avian spirochaetosis, secured the organisms using the Galloways technique (1925) with a simple modification by adopting a cotton wool plug soaked in paraffin to prevent evaporation. The author tried the method after Kligler et al. (1938) in the cultivation of borreliae concluding that the both methods are unreliable, as the organism usually lost its virulence after the second subculture and the probable contamination was quite difficult to be avoided. He maintained his isolates on citrated blood kept in the refrigerator at 4°C and serially passaged through fowls once every three weeks. He observed that sodium citrate may had a harmful effect on the organism concluding that it is better to use defibrinated blood instead of the citrated one. After that he stated that the vigirous shaking of the glass beads during the defibrination process may affect the delicate organism concluding that the most reliable method for

its maintenance was to leave the infected blood to clot then the separated serum was kept in the refrigerator at 4°C. He found that the spriochaetes remained alive and virulent for at least 30 days.

Morcos et al. (1946) isolated their strains in infected fowl serum kept in the ice chest for at least four weeks. They mentioned that the virulence of borreliae was lost when it was isolated in citrated whole blood.

McKercher (1950) obtained borreliae by its cultivation in embryonated eggs via yolk sac technique concluding that this method is the simplest and reliable method for the propagation of such organism.

Hart (1963) isolated Borrelia anserina in citrated blood stored in the refrigerator. He stated that by this method borreliae could stand alive and virulent for four months.

Gross and Ball (1964) isolated their strain in citrated blood kept in the refrigerator at 4°C and serially passaged it in Rhode Island Red hens once every two weeks.

Lotfy et al. (1966) maintained their strain in citrated blood and they mentioned that it could be

kept alive and virulent for 118 days at 4°C. They found that borreliae kept in citrated blood by freezing at - 20°C were avirulent concluding that the last method for its maintenance was unsatisfactory.

Al Hilly (1969) isolated Borrelia anserina in a citrated blood kept in the refrigerator at 4°C and he mentioned that the organism can grow by this method for at least 38 days.

Djankov and Soumarov (1973) preserved Borrelia anserina in liquid nitrogen for 120 days, the citrated blood containing spirochaetes was firstly frozen by being placed in wells or ampoules and then cooled by solid carbon dioxide.

Farid et al. (1980), in their studies on Borrelia anserina, obtained their isolates in citrated blood (one part of 4% sodium citrate + 4 parts of infected blood), from different localities scattered all-over Sharkia Governorate. All these isolates were kept in the refrigerator at 4°C and serially passaged in Dokki fowls once every three months. They reported that the aforementioned method is the best as the cultivation trials were futile for the isolation of the organism.

II- Antigenicity and Immunization:

The literature dealing with this heading contains very scanty informations and the recorded findings appear to be incomplete and undecisive.

Gabritschewsky (1898) reported the presence of spirochaeticidin antibodies in the serum of geese recovered from an infection with Borrelia anserina and this bactericidal power was destroyed by heating of this serum to 60°C. He furtherly found that blood contained not only spirochaeticidins but also agglutinins and lysins which were responsible for the clumping of the organisms.

Marchoux and Salimbeni (1903) prepared a synthetic vaccine by mixing virulent blood with specific antiserum. He found that the spirochaetes were dead and when this mixture was injected into fowls twenty four hours before infections, it prevented the disease.

Neufeld and Prowazek (1907) found that the disease was prevented by injection of 0.0025 cc of immune serum into fowls twenty-four to forty-eight hours before infection. They added that virulent blood containing spirochaetes killed by addition of sodium taurocholate might be useful as a vaccine.

Blaizot (1909) recorded that young and old chickens behaved differently against the infection with the same original strain of spirochaetes. The passaged strain in young chicks could not immunize old chickens against the infection with the same strain when passaged through old chickens, but the old chickens could be immunized against both strains.

Bonet (1909) mentioned that there was cross immunity between the Brazilian and the African strains of spirochaetes.

Dschunkowsky and Luhs (1909) obtained a curative and preventive vaccine from the serum of recovered geese and they said that injection of 0.01 g. of this serum protected against the disease while 0.5 g. proved to be curative.

Aragao (1911) prepared a vaccine from infected fowl blood. The spirochaetes in this vaccine were rendered motionless by its treatment with formalin (35.2% HCHO). He gave a single dose of 1 cc subcutaneously to attain a protection against infection and he mentioned that it retained its efficacy for 13 months.

Aoki (1914) found that the antigenic properties of spirochaetes were not injured by drying at 50°C

for 24 hours, thorough washing with saline followed by refrigeration for a month or by treatment with chloroform.

Hoffman (1921) reported that the blood of recovered birds was guarded against the infection for three to four weeks and he mentioned that there was a hereditary transmission of the immunity in chickens.

Verge (1923) successfully immunised fowls with infected blood which had been kept for sixty days or heated at 55°C for 5-10 minutes.

Knowles et al. (1932) reported that the injection of 2 ml. of immune serum twenty four hours before infection was recommended for the prophylaxis against this disease. They reported that the birds acquired a strong immunity but they failed to mention the duration there.

Morcos (1935) prepared a vaccine by emulsifying the liver and spleen of infected birds in glycerine saline then preserved it in formalin and kept for ten days.

Kligler et al. (1938) prepared a formalized vaccine as well as immune serum plus live spirochaetes

to protect fowls against this disease, they found that it gave immunity lasting for six months. They reported that four strains were obtained from five different districts in Palestine and all these strains were serologically variant. They also found that the chickens immunized with a given strain were definitely immune to the infection with the homologous strain, but remained susceptible to an infection with a heterologous strain.

Sreenivasan and Sankaranaryan (1943) reported that the chemotherapy after a natural infection was the best and safest method of immunization. They recorded that the suitable dose of the chemotherapeutic agent irrespective of breed, age and body condition was 0.015 - 0.02 gm of salversan given subcutaneously and they mentioned that the induced immunity was lasting for 8 months.

El Dardiri (1945) prepared separately three types of spitochaeta vaccine i.e. Blood vaccine, liver and spleen vaccines and he kept them in a refrigerator after addition of carbolic acid for one week before their administration. He mentioned that these vaccines proved to be of high protective value which may reach 100%. He concluded that a mixture of the three

vaccines must be used to give satisfactory results. He also reported that immunization of fowls by inoculation with infected blood and immune serum either simultaneously or twenty four hours before the infection proved very efficient and the immunity acquired by this method lasted for 13 months.

Morcos et al. (1946) found that a phenolised suspensions of spleen, liver and blood of the infected fowls to be effective in immunising birds injected with two doses at 5 days interval.

Gorrie (1950) used first a phenolised and later a formalised vaccines prepared by chick embryo inoculation via yolk sac route of 6 days old embryos and further incubation for 7 days and then harvested for the vaccine preparation.

Henry (1950) used a formalised vaccine prepared after inoculation of chick embryo of 12 days old via the allantoic cavity then furtherly incubated for 5 days and after that harvested for vaccine preparation. He stated that the vaccine prepared by this technique set up an immunity within 3 days.

Rao et al. (1954) pointed out the probable excistence of antigenically variant strains of Borrelia anserina and its importance in massive vaccination of

poultry flocks against fowl borreliosis. They also prepared a formalized chicken embryo vaccine of good results in immunization gagainst this disease.

Sohrab et al. (1957) prepared spirochaeta vaccine by inoculation of chicken embryos via yolk sac route. They reported that the isolated strains from Iran were antigenically identical.

Rao and Gupta (1960) isolated four strains of Borrelia anserina from different localities in India. These strains were antigenically identical. The strains were Nagpur Strain, Delhi strain, Izatnagar A and Izathnagar B strains.

Sabry and Sheble (1960), in Egypt, prepared a spirochaeta vaccine by the inoculation of chicken embryo of 6 - 8 days old and furtherly incubated for 5-7 days then harvested for the vaccine preparation. They added 30 ml. of 1% formalized saline for each embryo and used it for vaccination of fowls. They stated that the given immunity was set up for at least 35 months.

Hart (1963) prepared an egg-grown vaccine via chorioallantoic sac route and he recommended a dose