

FOWL SPIROCHAETOSIS IN IRAN WITH SPECIAL REFERENCE
TO CONTROL THE DISEASE BY IMMUNIZATION WITH
A FORMOLIZED CHICK-EMBRYO VACCINE

By

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Fowl Spirochaetosis is one of the most wide-spread poultry diseases in IRAN, and is of great economic importance. Although the incidence of the disease and its mortality rate are much higher among foreign than native breeds, the indigenous birds are also susceptible to the disease. Most of the affected native birds, possibly because of some inherited immunity, show the mild form of Spirochaetosis which is sometimes symptomless and ends in complete recovery without treatment.

Climatic conditions of IRAN favour the propagation of fowl tick «*Argas persicus*» which is wide-spread all over the country. Since intensive poultry breeding in the modern concept is uncommon and nearly the whole poultry population of the country is raised by farmers under primitive poultry husbandry practices the idea of tick eradication in such conditions seems impracticable. Therefore it was of interest to study the problem and produce a potent vaccine which could be used as a prophylactic agent in combating the disease.

Etiology : The causal agent of Fowl Spirochaetosis is *Borrelia anserinum* (Sakharoff 1891) which was first described in Caucasia among infected geese (1). Since then the disease has been diagnosed in all parts of the world among chicken, turkeys, pigeons, guinea-fowls, canaries and pheasants. In IRAN the disease was first diagnosed by RAZI-Institute's workers near Hessarek; then it was found that the disease was wide-spread in all parts of the country among chicken and turkeys.

The organism is named by different authors as : Spirochaeta

anserina (Sakharoff 1891), *S. gallinarum* (Stephence & Cristophers 1904), *Borrelia gallinarum* (Swellengrebel 1907), *Spirochaeta neveuxi* (Brumpt 1909), *S. granulosa penetrans* (Balfour 1911), *S. gallinarum* var. *hereditaria* (Neumann & Mayer 1914) and *S. analis* (Parrot 1920) (2) but it does appear that they are all synonyms for the same causative agent. The organism is 7-30 μ . in length by 0,3 μ , in width with 5-15 undulation. The unicity of different fowl Spirochaete strains found in various countries and hosts is the object of contradictory statements in the literature (3.4.5.6.7.8.); but in this country as we will see later, 5 strains of *B. anserina* isolated from different parts of IRAN (HESSARAK, MESHED, ZARKASH, MAMAZAN, ZAHAND) are antigenically identical.

B. anserinum can be cultivated in different artificial media developed by Noguchi (1912), Ungermann (1918), Galloway (1926), Chorine & Marchoux (1931) and Kligler & Robertson etc. (8.9). Some of the above media have been used in this Institute; however, regular subculturing is impracticable.

Mode of infection : The disease is transmitted from bird to bird primarily by *Argas persicus*, and *Argas reflexus* (6); however, different external parasites such as : Red mite «*Dermanyssus avium*» (10), mosquitoes «*Culex*» (11) and lice «*Menopon pallidum*» are also incriminated as transmitting vectors. In this country we believe that *Argas persicus*, due to its wide-spread propagation, is the most important intermediate host. Although we could experimentally transmit Fowl spirochaetosis by *Argas reflexus* and even though this tick occasionally lives in poultry house in IRAN, its role in natural infection is questionable. Work has been done in this Institute and elsewhere (8) showing that hereditary transmission of *B. anserinum* through infected mother tick (*Argas persicus*) to its progeny helps to preserve and spread the infectivity of ticks in nature. Besides the disease transmissions through vectors; susceptible birds can be directly contaminated by eating infected ticks or their eggs or ingestion of materials from sick birds (12.18.29.32.33.).

Symptom and post-mortem finding : The incubation period in experimental transmission, by infected ticks (*Argas persicus*) and (*Argas reflexus*), varies from 4-13 days; however, after injecting infected fowl blood into healthy susceptible chicks, this period can be as short as 1-4 days depending upon the route of inoculation, the age of susceptible chicks and the number of organism in the injected material. Moreover, we have noted that for successful transmission, the infected blood should be taken in the early stage of the disease otherwise the results may be irregular. The symptoms and post-mortem

lesions of the disease are the same as those described in available literature such as : loss of appetite, rise in temperature, dullness, greenish diarrhoea, lameness, pale and yellowish comb and wattles and slight or marked enlargement of the spleen and liver; these symptoms are more severe among the foreign breeds than the indigenous birds.

Treatment : Different arsenical compounds such as : Atoxyl, Neosalvarsan, Nekharsivan, Soamin, Mapharsen, Myosalvarsan and Khar-suphan are successfully used by intravenous or intramuscular injections (8.12). Good results have also been reported by some authors using penicillin for this purpose (13.14.15.). In the early stage we have found that either Novarsenobenzol or penicillin-therapy is effective against field and experimental cases of fowl spirochaetosis.

Control : Eradication of ticks and other external parasites with insecticides is the main control measure to be applied; however, due to poor conditions of poultry husbandry (as previously mentioned) tick control is rather difficult in this country.

Immunization

Review of Literature : After Levaditi's findings proved the existence of specific antibody in the serum of fowl recovering from spirochaetosis, early literature showed that spirochaetal antiserum, prepared from geese, goats, donkeys and horses, can be used for passively immunizing birds (8); attempts to prepare vaccines by heating the infected blood (5-10 minutes at 55°C.), keeping virulent blood at room temperature for 48 h. or using dissolved spirochaetes with sodium taurocholate or antiformin, have been made by some workers (16). Marchoux et al; (17) described a vaccine which consisted of infected fowl blood mixed with spirochaetal antiserum. Aragao (18) prepared a vaccine by formalizing virulent blood which he claimed would confer immunity in birds in a vaccinal dose of 1cc. with the vaccine retaining its potency for about 13 months. Kroo (19) showed that the antibody forming pattern was different in adult and young birds which were experimentally infected; he could not immunize young chicks with killed spirochaetes. Morcos (20) prepared a vaccine by emulsifying and formalizing infected fowl's liver and spleen. Kroo (21) mentioned that egg whites and yolks as well as the sera of corresponding embryos from recovered birds contained some spirochaetal antibodies. Toth (22) could protect 64 adult and 35, six months old, birds with antiserum and virulent blood. Kligler (17) used a formalized

culture vaccine as immunizing agent in the dose of 5cc. and claimed 6 months immunity in vaccinated fowl; he also used, immune serum (0.5cc.) followed by injection of defibrinated virulent blood (0.25cc.). Nobrega (23) by taking advantage of Schafer's work (24) on *B. anserinum* cultivation in embryonated eggs; prepared the first chick embryo formalized vaccine which in the dose of 0.5cc. conferred a solid immunity in chicken. Pires (25) produced a similar vaccine by using infected embryos, amniotic fluid, chorio-allantoic membrane, lungs, liver and heart. Sreenivasan (26) by injection of Sulpharsenol in 15 milligram dose followed by inoculation of infected blood protected birds for a period of 8 months. Nobrega (27) mentioned that antibodies from the vaccinated fowl could be transmitted to the offsprings 10-16 days post vaccination. Morcos & Coll. (28) described three procedures for immunization: (a) carbolized tissue vaccine; (b) infecting susceptible birds followed by arsenical treatment, (c) antiserum injection followed by experimental infection; they claimed that these three procedures immunized chicken for at least 6 months. Gorrie (7 & 29) in preparing chick-embryo carbolized and formalized vaccines against the disease claimed that carbolized vaccine conferred a short period immunity while the formalized vaccine in 0.5cc. dose protected fowls for one year. Rao (15) made a chick-embryo formalized vaccine with a strain of *B. anserinum* which was kept for 20 years in Moktesvar laboratory of INDIA. Dixit (30) by keeping infected fowl blood for 14-21 days in refrigerator, then adding penicillin to it at the rate of 20,000 U. per cc. prepared a vaccine which was effective as a prophylactic agent. Pearson (31) mentions a chick-embryo formalized vaccine made by L. Hart in New-South Wales (Australia) from which 35,000 doses were successfully used in field vaccinations up to 1954; this vaccine in the dose of 1cc. protected birds for about 18 months.

EXPERIMENTAL

The procedure adopted by us for preparing experimental chick-embryo vaccine is a modification of the technics used by others.

Maintenance of the strain for vaccine production: Infected defibrinated, citrated or clotted serum extracted blood is used by other workers for this purpose (15.26.28.29); by these methods they have been able to keep the organism alive up to 54 days. In our experiments we found that the clotted whole blood from infected birds keeps the spirochaetes alive and virulent much longer than any other

procedures. With this material we can infect 7 days old chick-embryo after 3 months of storage at +4°C. (the longest period checked); therefore, for maintenance of the strain we bleed the infected bird aseptically by wing vein or heart punctures; then the virulent blood is dispensed in several sterile cotton plugged agglutination tubes in the amount of 2CC. in each and then stored at +4°C. The alternate way of preserving *B. anserinum* is by feeding laboratory bred *Argas persicus* on experimentally infected birds. When needed, these ticks are fed again on or injected into susceptible birds to get the organism from the blood of these tick-infected chicken. Attempts to keep fowl spirochaetes by lyophilisation of infected blood were unsuccessful.

Vaccine production: Either fresh citrated or stored clotted whole virulent blood are used for chick-embryo inoculations. Where clotted blood is used 1CC. of physiological saline is added to each of the agglutination tubes as mentioned and the clot is emulsified in saline with a sterile Pasteur pipette; the resulting liquid is used as inoculum. Embryonated eggs 7-8 days old are injected into the yolk sac by either one of the above materials, the dose of inoculum being 0.1cc. After 7-8 days re-incubation at 37°C. all the live embryos are selected for vaccine production. These eggs after being chilled for 6 hours at +4°C. (to contract the blood vessels and prevent bleeding from the C. A. membrane incision during the harvesting process) are opened and the embryos +C.A. membranes, representing a high concentration of spirochaetes, are harvested aseptically. These materials are ground up twice in the Atomix blender each time for one minute. Thirty cubic centimeters of a 1% formalized physiological saline for each embryo is added and then blended for an additional one minute. The resulting mixture is kept in a sterile jar at +4°C. for 2 days. This suspension after being filtered through sterile gauze, diluted with 1% formol saline to twice of its original volume and checked for sterility, is ready to be used as a vaccine.

Vaccination trials;

a) Laboratory tests of the vaccine were performed on 38 White Lohorn birds, 3-4 months old (see table I and III); these birds were vaccinated with a vaccine dose of 1cc. via the intramuscular route into the pectorals and 12-29 days after vaccination were challenged along with 15 controls (table I & III) with 0.5cc. of virulent blood which contained an average of 10 organisms in each microscopic field of dark field illumination. Blood smears made from these birds were tested daily up to 15 days. All the controls except one showed disease symptoms and spirochaetes in the blood

TABLE I

N° of Exp.	N° of Chicken		Dose of vaccine	Interval before challenge	Challenge		Results up to 15 days post challenge	
	Vaccinated	Control			Material	Dose		
1	4	—	{ 1 cc.	{ 29 days	Virulent blood representing an average of 10 organisms in each microscopic field	0,5cc	{ Negative	
	7	—				»		
2	—	3	{ 1 cc.	{ 12 days	» »	»	Positive	
	11	—				»	{ Negative	
	to 26	—				»		
	—	27				—	»	Positive
	—	28				—	»	Positive
		29				—	»	Positive
	30	—	—	»	Negative			

Negative= No spirochaetes found in the blood smears and no symptom noticed

Positive= Spirochaetosis symptoms noticed with presence of *B. anserinum* in the blood

within 48-72 hours post-challenge but none of the vaccinated birds showed any untoward symptoms or spirochaetes in the blood. The results are tabulated in tables I and III.

b) Field trials: 1800 doses of the vaccine were delivered to 3 poultry breeders around Tehran. All 3 flocks were infested by *Argas persicus* and several cases of spirochaetosis were diagnosed among these birds before the vaccination trials. In the first flock which consisted of 900, six months old pullets, 800 were vaccinated in July 1957 and 100 kept as non vaccinated controls. According to the owner's report, the disease stopped among the vaccinated birds at one week post-vaccination while among the controls up till the present time 12 out of 100 showed typical symptoms of spirochaetosis and 9 out of these 12 sick birds responded to penicillin-therapy. In the other 2 flocks, where fowl spirochaetosis caused considerable losses each summer, no cases of the disease were reported after vaccination.

Duration of immunity in vaccinated birds: One test has been conducted in the laboratory on 2 vaccinated chicks to determine the duration of immunity. These 2 birds have shown a solid immunity for a period of 4 months. To obtain further information on the duration of immunity, we have recently vaccinated 50 White Loughorns, four months of age, in the laboratory and also arranged to purchase some of the field vaccinated birds to be challenged at regular intervals; the result of these experiments will be discussed in a future report.

Identity of different strains of B. anserinum in IRAN

In order to determine whether or not immunogenic differences existed between different strains of fowl spirochaetosis in Iran and if a vaccine prepared by a given strain of *B. anserinum* could be used for the entire country, five strains of the organism were checked by cross-immunity test. The results of this test as shown in table II & III indicates that for prophylactic purposes these five strains are antigenically identical and have good cross-immunizing properties.

The cross-immunity tests are to be repeated as new strains become available to confirm the unicity of *B. anserinum* strains originating from all parts of the Iran.

Conclusion:

- 1) fowl spirochaetosis which is a wide-spread Poultry disease, is describe in IRAN.
- 2) The chick-embryo formolized vaccine prepared in Razi

TABLE II

N° of birds	Infection by different strains of <i>B. anserinum</i>		Interval between the two infections	Reinfection by Hessarek strain of <i>B. ans.</i>		Results
	Origin of the strain	Mode of infection		Material	Dose	
112	Mamazan	Natural (treated by Penicillin)	12 days	Virulent blood representing 7-10 organisms in each microscopic field	0,5cc	Blood smears examined daily up to 10 days post reinfection.
113	"					
116	Zarkash	Experimental (by infected <i>Argas persicus</i>)	24 days	"	"	No Spirochaetes found " "
124	Meshed					
110 111	Control					

TABLE III

N° of Exp.	N° of chicken		Interval before challenge	Challenged by different strains of <i>B. anserinum</i>		Results up to 10 days post challenge
	Immunized with Hessarek strain vaccine in 1cc. dose	Control		Origin of the strain	Material & Dose	
3	31,32,33,34	— 64 & 65	15 days	Zarkash »	0,5cc of virulent blood showing 6-12 organisms in each microscopic field	Negative. Positive.
	35,36,37,38	— 66 & 67	15 days	Meshed »		Negative. Positive.
	39,40,41,42	— 68 & 69	15 days	Mamazan »	» » » »	Negative. Positive.
	43,44,45,46	— 70 & 71	15 days	Zarand »	» » » »	Negative. Positive.
	47,48,49,50	— 72 & 73	15 days	Hessarek (vaccine strain)	» » » »	Negative. Positive.

Negative = No symptoms noticed in birds and no spirochaetes found in the blood.

Positive = Spirochaetosis symptoms noticed with presence of *B. anserinum* in the blood.

Institute seems to be an effective prophylactic agent to control the disease.

3) Clotted whole virulent blood stored in $+4^{\circ}\text{C}$. preserves the virulence of *B.anserinum* for longer than 3 months.

4) The unicity of 5 different *B.anserinum* strains originated from various parts of the country is confirmed by cross-immunity tests.

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SOMMAIRE

1) La spirochaetose des volailles, maladie très répandue en IRAN avec une grande importance économique, est étudiée.

2) Le vaccin formolé préparé sur l'embryon de poulet à l'Institut Razi semble avoir une action prophylactique très efficace contre la maladie.

3) L'unicité de 5 différentes souches de *B.anserinum* provenant de diverses régions du pays est confirmée par l'immunité croisée.

4) Le sang coagulé des poules infectées qui est conservé à la glacière de $+4^{\circ}\text{C}$. preserve sa virulence plus de trois mois.

REFERENCES

- 1) Sakharoff, N. (1891) - *Spirochaeta anserina* et la septicemie des oies; Ann. Inst. Pasteur, 5,564.
- 2) Neveu-Lemaire, M. (1943) - *Traité de protozoologie médical et vétérinaire* p: 735.
- 3) Verge, J. (1936) - Les spirochaetoses aviaires, Rec. Med. Vet. 112 : 257-270.

- 4) Kligler, I. J. & Coll. (1938) - Studies on Fowl Spirochaetosis, presence of serologically differentiated types; *J. Comp. Path.* 51 : 206-212.
- 5) Carpano, M. (1938) - Poultry Spirochaetosis in Albania, the cycle of the spirochaetes in intervertebrated vectors; *Vet. Bull.* 11 : 438.
- 6) Kamberis, E. (1937) - la spirochaetose des poules, thèse Alfort résumé in *Vet. Bull.* 9 : 14.
- 7) Gorrie, C.J.R. (1952) - Vaccination against spirochaetosis in Fowls; *Aust. Vet. J.* 28 : 155-156.
- 8) Lesbouyries, G. (1941) - La pathologie des oiseaux; 579-590.
- 9) Calmette, A. & Coll. (1948) - Manuel technique de microbiologie et de Sérologie, 533.
- 10) Hungerford, T. G. (1937) - Fowl tick fever (spirochaetosis) also transmitted by common red mite; *Agric. Gaz. N. S. W.* 48 : 591-592.
- 11) Zuelzer, Margarete (1936) - *Culex*; a new vector of spirochaeta gallinarum; *J. Trop. Med. (Hyg.)*, 39 : 204
- 12) Hungerford, T.G. (1951) - Disease of poultry, 212 & 215.
- 13) Nobrega, P. & Coll. (1945) - On the treatment of avian spirochaetosis with Penicillin; *Vet. Bull.* 17 : 183.
- 14) Bornstein, S. & Coll. (1954) - The therapeutic use of parenteral and oral Penicillin in Fowl Spirochaetosis, Xth. World's Poultry Congr. 262-265.
- 15) Rao, S.B.V. & Coll. (1954) - Studies on Fowl Spirochaetosis with special reference to Penicillin-therapy; *Indian Vet. J.* 31 : 1-14.
- 16) Kligler, I. J. & Coll. (1938) - Studies on Fowl Spirochaetosis; *J. C. Path.* 51 : 197.
- 17) Marchoux, E. & Coll. (1903) - La spirillose des poules; *Ann. Inst. Past.* 27 : 569.
- 18) Curasson, G. (1943) - Traité de protozoologie vétérinaire et comparé 2 : 72 & 76.
- 19) Kroo, H. (1934) - Studies on immunity and chemotherapy in new born and adult animals, Spirochaetal infection in Fowls; *Vet. Bull.* 5 : 654.
- 20) Morcos, Z. (1935) - Preliminary studies in Fowl Spirochaetosis in Egypt; *Vet. J.* 91 : 161.
- 21) Kroo, H. & Coll. (1935) - Inherited immunity of Fowl Spirochaetosis *Vet. Bull.* 9 : 534.
- 22) Toth, A. (1938) - Research into Spirochaetosis of Poultry; *Vet. Bull.* 10 : 87.
- 23) Nobrega, P. & Coll. (1941) - A vaccine against Fowl Spirochaetosis based on the cultivation of *Sp. gallinarum* in the chick-

- embryo; Vet. Bull.; 12 587.
- 14) Schafer, W. (1939) - The maintenance of Sp. Gallinarum by passage in Fowl embryos; Vet. Bull. 11 : 220.
 - 25) Piers, A. (1942) - Vaccination against avian Spirochaetosis with chick-embryo vaccine; Vet. Bull. 13 : 207.
 - 26) Sreenivasan, M. K. & Coll. (1943) - Spirochaetosis of Fowls in INDIA; Vet. Bull. 14 : 52.
 - 27) Nobrega, P. & Coll. (1945) - On the Immunity of Cicks and Chik-embryos form hens vaccinated against Fowl Spirochaetosis; Vet. Bull. 17 : 183.
 - 28) Morcos, Z. & Coll. (1946) - A concise investigation of Fowl Spirochaetosis in EGYPT; J. Amer. Vet. Med. Ass. 109 : 112.
 - 29) Gorrie, C.G.R. (1950) - Vaccination against Spirochaetosis in Fowls; Aust. Vet. J. 26 : 308-315.
 - 30) Dixit, S. G. (1955) - Note on a method of protecting Fowl against Spirochaetosis; The Indian J. Vet. Sci. & Animal husbandry, Vol. XXV Part I March.
 - 31) Pearson, I. G. (1954) - Personnal communication.
 - 32) Hoffman, H. A. & Coll. (1946) - Spirochaetosis in turkey; J. Amer. Vet. Med. Ass. 109 : 481-486.
 - 33) Mc Neil, E. & Coll. (1949) - A study of Borrelia anserina infection (Spirochaetosis) in turkeys; J. Bact. 57 : 191-206.