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STATUS OF MYCOPLASMA SYNOVIAE IN CHICKENS IN UPPER EGYPT

(With 4 Figs.)

By

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(Received at 17/6/1989)

صورة الميكوبلازما ساينوفى في الدواجن بصعيد مصـــــــــــــــــــــر

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أوضح الفحص السيرولوجى لقطعان الدواجن من مختلف الأعمار ومن مناطق مختلفة بصعيد مصر الإصابة الحادة بالميكوبلازما ساينوفى · تم عزل م · ساينوفى من الحنجرة ، الأكياس الهوائيـــة ، المفاصل ، البيض · وتم تصنيف الميكروب بيوكيميائياً وسيرولوجياً ثبـــت أن م· ساينوفى المعزولة أنها ضارية للأجنة البيض وتسببت في حدوث ٧٥٪ وفيات في الأجنـــة مع ظهور الأعراض المميزة للميكروب في الأجنة · كذلك تم إحداث المرض في كتاكيت عمـــر يوم عن طريق الحقن في الحنجرة ووسادة القدم · ظهرت الأعراض والآفات التشريحية للمـــــرض خلال فترة الملاحظة التى إستمرت ٤ أسابيع · كما تبين ظهور الأجسام المناعية بعد العـــــدوى بأيام قليلة وذلك باختبار التلازن السريـــــع ·

SUMMARY

Serological screaning of chickens of various ages and different localaties in area of Upper Egypt revealed high incidence of M. Synoviae infection.

M. Synoviae was isolated from trachea, air-sacs, joints and yolk. The organism was identified biochemically and serologically by FA test.

The recovered isolates of (MS) were highly pathogenic to chicken embryos causing 75% mortality and characterstic lesions in embroys.

Also it was pathogenic to one day old chicks when experimentally infected through tracheal and Foot-pad inoculation. Characterstic symptoms and lesions were observed with 4 weeks observation period, antibodies were detected as early as few days (PI) by RSA.

INTRODUCTION

Most of the economic losses in Poultry are related directly or indirectly to mycoplasmas infections with or without complicating factors.

In the last few years extensive studies were published on mycoplasma infections

in Poultry in A.R.E., although the incidence and role of mycoplasmas have been described in different species of birds, but there are no informations about Mycoplasma synoviae infection particularly in the area of Upper Egypt.

Infection with M. Synoviae (MS) may cause disease in wide variety of organs and tissues (KLEVEN, et al. 1975), its effect on joints was first described, in chickens by (OLSON, et al. 1964). M. Synoviae was first recognized and described by FABRICANT (1960). The role of MS in causing air-sacculitis, respiratory distress and infectious sinusitis is now apparent GOREN, et al. 1975.

Under natural conditions, it is probable that clinical disease may be seen in one or both of two forms: lamness with or without generalized disease and respiratory form, KLEVEN, et al. 1972.

Serological examination by RSA and (HAI) tests is used to indicate the infection status of a flock. The HAI test is generally considered to be more specific, than RSA, unfortunataly it is less sensitive and develops 7-10 days later than the plate test, KLEVEN and SOLIMAN, 1988.

M. Synoviae spreads mainly by direct means through the egg (VARDAMAN and YODER, 1970) and through droplet particles from the respiratory tract (OLSON, et al. 1965).

The objectives of the present studies are determination of the incidence and role of M. Synoviae infection in chickens at the area of Upper Egypt.

MATERIAL and METHODS

I- Serological screening of poultry farms:

Two hundred serum samples were collected from different ages and different localaties in area of Upper Egypt. The sera were tested against M. Synoviae stained antigen, a product imported from Holland (Intervet), by the Rapid Serum Plate Agglutination test (RSA) after ADLER, (1954). The positive sera with RSA were subjected to micro-haemoagglutination inhibition test (m-HAI) after YODER, 1970. HAI antigen for (MS) was kindly supplied by Prof.Dr. S.H. Kleven, Athens, Georgia, U.S.A.

II- Isolation and identification of M. Synoviae:

Tracheal, air-sacs, joint and yolk swabs were collected from dead and sacrificed birds of various ages and different localities in Upper Egypt. Swabs were directly inoculated in Brain-heart infusion broth medium, suplemented with 1% Nicotinamide adenine dinucleotide (NAD Sigma) and 15% Swine serum (collected from Assiut Abatoir, steralized by Filteration and Stored at -20°C), broth inoculated cultures were incubated aerobically at 37°C for 3 days.

Subculture were performed on BHI-agar supplemented with NAD and Swine Serum, plates were incubated at 37°C in a moist container with low oxygen tension.

After three days plates were examined by using dissecting microscope for the presence of the common Fried-egg Colonies.

The culturing technique was carried out after (SABRY, 1968). The recovered isolates were maintained for three pass-ages on BHI-agar without inhibitors to exclude bacterial reversibility. The suspected colonies were subjected to: Digitonin-Sensitivity test by running-drop technique after FREUNDT, et al. (1979) and examined biochemically for glucose-Fermentation and Arginine hydrolysis (ERNO and STIPKOVITIS, 1973). The immuno-Fluorescent antibody test was applied directly to colonies on agar, after AL-AUBAIDI and FABRICANT, (1971) for serological identification of isolates, (Standared strains, antisera, and Fluorescein-conjugated antirabbit immunoglobulin were supplied by Prof. Dr. S.H. Kleven, University of Georgia Athens, G.A., U.S.A.).

III- Experimental infection:

1) Inoculation of Embryonated chicken eggs:

A total of 70, Fayomi chicken eggs were obtained from Poultry Farm of Agriculture College, Assiut University. Ten eggs were examined for maternal antibodies by Egg-yolk agglutination test after (BENJAMIN, 1961) and for isolation of mycoplasmas, eggs proved to be free from mycoplasmas organisms or antibodies.

Sixty eggs were incubated for 5 days, then classified into 3 groups (20 of each).

Group I:

Eggs were inoculated via yolk-sac with 0.2 ml of three days old broth culture of isolated strain ($2x10^7$ CFU/ml).

Eggs of Group II:

Were inoculated by the same route with 0.2 ml, 3 days old broth culture of reference strain ($2x10^7$ CFU/ml).

Group III:

Were inoculated with 0.2 ml of Sterile broth and used as control group. The used broth medium was BHI broth with all suplements except inhibitors.

Eggs were examined daily, deaths were recorded and dead embryos were subjected to p.m. examination and reisolation of the organism.

2) Reproduction of the disease in chickens:

100, Fertile chicken eggs (Hubbard) were obtained from the national Poultry Co., eggs were incubated at 37°C for one day then dipped in 2-6°C solution containing (7 gm tylosin tartarate and 5 gm lincomycin for 10 litter water), then eggs were

incubated tell hatching. Ten, one-day old chicks were slaughtered and proved to be free MS organisms. A total of 75, one-day old chicks were divided into 5 groups (15 bird of each).

Group A: inoculated intra-tracheally.

Group B: inoculated in foot-pad.

The two groups were inoculated with 0.5 ml of 3 days old broth culture of isolated strain ($2x10^7$ C.F.U/ml).

Group C: were inoculated intra-tracheally and Group D: injected in Foot pad by using 3 days old broth culture of reference strain ($2x10^7$ C.F.U/ml).

Group E: Kept as non-infected control group.

All groups were kept in cages, in separate places under the same environmental conditions. The observation period continued for 4 weeks. Clinical signs and p.m. lesions of dead birds were recorded. Serum samples were collectd weekly and examied by RSA test. Tracheal swabs were cultured weekly on BHI-broth media nad results of MS reisolation were recorded. At end of the experiment all birds were slaughtered, necropsied and samples of trachea, air- sacs and tendons were taken directly in ice-bags, processed, sectined at 6 microns, washed in buffer saline (PH. 7.2), then covered on a glass slide with fluorescein-conjugated antirabbit immuno-globulin against MS and examined under Fluorescein microscope after PETERS, et al. (1966), positive and negative slide were included.

RESULTS

The results of the serological screening are illustrated in Table (1).

Results of isolation and identification of M. Synoviae from different ages and localates in Upper Egypt are tables in table (2).

Results of experimental infection:

1) **M. Synoviae in chicken embryos:**

Results of embryonic mortalaties are illustrated in table (3). Dead embryos in both isolated and reference strains appeared congested, odematus, enlarged liver with haemorrhagic patches and necrotic areas, swollen joints and turbid greyish embryonic fluid. The inoculated strains were successfully reisolated from all inoculated eggs.

2) **M. Synoviae in one day-old chicks:**

Results of the experimental infection of one-day-old chicks proved that both isolated and reference strains were pathogenic. The clinical manifestations in groups inoculated via food-pad were observed after 7 days post-infection (PI) while respiratory signs observed in groups inoculated Intratracheally within 5th day (PI).

Group A and C revealed at 5th day PI general depression, loss of appetite, nasal discharge, wet-eyes, coughing sneezing, and rales. At 15th day PI birds showed sever respiratory signs and apparent swelling, hotness, redness of foot-pad and hock-joints. 21th day (PI) till end of the experiment birds showed respiratory signs peristant laimness and general emaciation and dehydration.

Group B and D, that experimentally infected via foot-pad, revealed at 7 days (PI) swelling, redness of foot-pad and hock joint, at 15 day (PI) mild respiratory signs, clear lamness and general depression. At 21 days (PI) respiratory signs poor growth, lamness and birds setting on hocks were observed.

The msot characterstic lesions at 7 days PI in group A, and C were slight tubidity of air-sacs and catarrhal trachitis. At 15 day (PI) dead birds revealed congestion of lungs, catarrhal trachitis; thickening and turbidity of air-sacs, in addition to creamy exudate involving foot-pad, tendon sheath and synovial membrane of joints. At the 4th w. (PI) the necrobsied birds revealed pericarditis, perihepatitis, ari-sacculitis and caseous exudate in joints.

Group B and D that inoculated via the foot pad showed at the first localized lesions involving foot pad and hock joint. At 21 day (PI) viscous, creamy exudate noticed in joints and foot-pad with slight turbidity of air-sacs nad catarrhal exudate intrachea. At 4th week (PI) the sacrificed birds showed orange, caseated material in foot-pad and joints, erosion of articular cartilage with turbidity and thickening of air-sacs and extensive exudate in trachea and bronchi.

The inoculated organisms were successfully reisolated from tracheal swabs of most inoculated birds. Results of deaths, resiolation and IFA test, are illustrated in table (4).

DISCUSSION

The results of the serological screening of chickens of various ages from differnet localities in area of Upper Egypt indicated that M. Synoviae is widly, and highly spreaded. High titers of HI-antibodies were detected in both broiler and breeder flocks also flocks revealed high degree of RSA-test positive had usually higher HI-titers. These results are in agreement with those of SAHU and OLSON (1975); SALEM, et al. 1986 and KLEVEN and SOLIMAN, (1988). Authors demonstrated antibodies in clams and offspring even though no clinical signs.

Although mycoplasmas had been frequently isolated in almost avian species in Upper Egypt, no studies were carried out on status of M. synoviae infection in this area. It is clear from the results of isolation and identification that M. synoviae constitute an important agent causing economic problem in flocks at Upper Egypt.

From table (2) it is clear that M. synoviae were recovered from most of the examined organs, this in agreement with the work of KLEVEN, et al. (1975). The successful recovery of MS from eggs of breeder flocks indicated the transmission of the organism through the egg, these results come parallel with observations of BRADBURY and KLEVEN, 1987, VARDMAN and YODER (1977).

Also its recovery from respiratory tract revealed its lateral spreading through droplet infections, these results in agreement with OLSON, 1965.

Results of experimental infection of embryonated chicken eggs, revealed that both of isolated and reference strains were highly pathogenic to chicken embryos causing 75 and 80% embryonic deaths respectively. The dead embryos within the last few days of incubation appeared oedmatous, dwarfed and congested, liver necrosis and joint abscesses were noticed. These results are in agreement with YODER and HOFSTAD (1965). The inoculated strains were positively reisolated from all dead embryos. During 4 weeks observation period of experimentally infected one-day-old chicks, via Intra-tracheal (IT) and foot-pad (FP) inoculation, it is clear that both isolated and reference strains were pathogenic to chicks.

The clinical signs were observed at the 5th day post-intratracheal inoculation and at the 7th day following foot-pad inoculation, these results are in agreement with YODER (1979).

Chicks inoculated (IT) with both isolated and reference strains revealed at the first mild respiratory distress and general weakness. After two weeks the respiratory signs progressed with lamness, and affection of joints. While birds inoculated via foot-pad showed at the first, signs of lamness and general weakness. At 15th day (PI) affection of foot-pad and joints become more clear and some birds died from starvation and thrist. At 21th day (PI) respiratory signs become more clear. These results indicated that isolated M. synoviae was pathogenic for chicks causing both respiratory and lamness signs and this come in agreement with GOREN, et al. 1975.

The most characterstic p.m. lesions were noticed clearely at the 4th week (PI) in all groups.

The inoculated strains were sucessfully reisolated from most of collected tracheal swabs but in very low incidence in groups inoculated via foot-pad.

Results of rapid-serum agglutination test revealed high positive reactors during the observation period, these results in agreement with VARDMAN and YODER, 1970 and SAHU and OLSON, 1975, the previous authors reported that the antibody response is first detected by RSA, test as early as few days after natural or artificial infection.

Results of direct immuno-fluorescien for detection of inoculated organism in tissues indicated that the organism was localized in both respiratory organs and joints producing inflammatory reactions and this goes parallel with observations of PETERS, et al. 1966 and ZIDAN, 1980.

Lastly depending up on the results obtained in this study, it could be stated that M. synoviae is widely spreaded in Upper Egypt, sharing with other mycoplasmas in producing highly economic losses and problems.

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Table (1): Results of Serological screening of sera for M. Synoviae antibodies.

Province / Farm	Breed	Age	No. of examined sera	Serological Tests/No. positive				Total No. of positive
				RSA	m-HI Test			
					1:40	1:80	>1:460	
Assiut	Arbor Acres							
		breeders	25	20	2	8	10	20
		broilers	20	11	2	4	5	11
El-Menia	Hubbard							
		layer	30	22	4	6	12	22
		broilers	25	18	2	6	10	18
Kenna	Lohman							
		breeders	20	12	1	3	8	12
		broilers	25	21	5	6	10	21
Aswan	Lohman							
		broilers	25	15	3	3	9	15
Sohag	Lohman							
		broilers	30	19	3	9	7	19
Total			200	138	22	45	71	138

Table (2): Results of M. Synoviae recovery from different ages and localaties.

Province / Farm	Breed	Age	No. of exam. samples				No. of recovered isolates			MS	Total No. of recovered M.S
			Trachea	Air/ sacs	Joint	Yolk	Trachea	Air/ sacs	Joint	Yolk	
Assiut	Arbor Acers	Breeder	20	20	20	15	3	3	1	3	10
		broiler	25	25	25	--	4	2	2	-	8
El-Menia	Hubbard	layer	15	15	15	20	3	2	2	2	9
		broiler	20	20	20	--	2	2	1	-	5
Kenna	Lohman	breeders	15	15	15	20	4	2	1	3	10
		broilers	10	10	10	--	3	1	1	-	5
Aswan	Lohman	broiler	20	20	20	15	2	3	3	2	10
Sohag	Lohman	Broiler	25	25	25	--	5	1	2	-	8
Total			150	150	150	70	26	16	13	10	65

Table (3): Results of egg-inoculation with isolated and feference M. synoviae.

Materials		No. of inoculated eggs	Deaths in Days Post-inoculation																Total No. of Deaths	%
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Isolated strain	$2x10^7$/ml	20	2	-	-	-	-	-	-	-	2	3	3	2	2	3	-	-	15	75
Reference strain	$2x10^7$/ml	20	1	-	-	-	-	-	-	2	2	4	3	1	1	3	-	-	16	80
Sterile broth	---	20	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2	--

Table (4): Results of Experimental infection in one-day old chicks.

Strain / Route of infection	No. of inoculated birds	Deaths				Risolation*				Serology				IFA test			
		1st w.	2nd w.	3rd w.	4th w.	1st w.	2nd w.	3rd w.	4th w.	1st w.	2nd w.	3rd w.	4th w.	1st w.	2nd w.	3rd w.	4th w.
Isolated strain																	
Group A I. Trached	15	2	1	1	-	11	6	6	5	6	10	6	8	2	1	1	8
B I. Foot pad	15	-	2	2	1	--	1	2	3	7	8	6	6	-	2	2	9
Reference strain																	
C I.T.	15	1	2	1	-	10	8	6	6	6	10	9	8	1	2	1	9
D I. Foot pad	15	-	-	2	1	--	2	2	3	8	10	8	8	-	-	2	9
E Control	15	-	-	-	-	--	-	-	-	-	--	-	-	-	-	-	-

* Positive reisolation from tracheal swabs.