Department of Mycoplasma
Animal Health Research Institute, Dokki ,Giza

EVALUATION OF SEROLOGICAL TESTS FOR THE DIAGNOSIS OF MYCOPLASMA GALLISEPTICUM IN COMPARISON WITH THE FREQUENCY OF ISOLATION.

(With 3 Tables)

By
Manal M. Abou El-Makarem

(Received at 18/9/2003)

تقييم الاختبارات السيرولوجية في تشخيص ميكوبلازما جاليسبتكم بالمقارنة بتكرار عزل الميكروب

منال أبو المكارم

تم تجميع عدد مائة وخمسون عينة سيرم من كتاكيت تعانى من الأمراض التنفسية من عمر يوم حتى عمر شهرين. تم إحداث العدوى الصناعية لعدد مائة وخمسون كتكوت عمر أسبوعين بالميكوبلازما جليسبتكم وقد أجريت الاختبارات السيرولوجية المختلفة علي السيرم. تم عزل الميكوبلازما جليسبتكم من الأعضاء التنفسية المصابة وقد وجد أن أعلى نسبة إصابة كانت من الحنجرة (٣٦%) ، يليها الأكياس الهوائية (٢٤,٧%) ثم الرئة (١٨%). وجد من الضروري إيجاد طريقة سيرولوجية سريعة سهلة ودقيقة للكشف عن الإصابة بالميكوبلازما فى المزارع. تم إجراء اختبار تجميع المصل على الشريحة (س.ب.م) وهو شائع الاستخدام في غرض عمل مسح من عمر يوم إلى أسبوعين بينما اختبار تلازن الدم المضاد (ه. ا) المستخدم فى التأكيد بعدوى الميكوبلازما ووجد ان استخدام اختبار الاليزا كاختبار تشخيص وقياس للأجسام المضادة فى دم الطيور ابتداء من عمر أسبوعين.

SUMMARY

One hundred and fifty serum samples were collected from chickens showing respiratory manifestation of different ages. (One day old until two months) from different localities (Cairo, Giza and Kaliobia governorates) and one hundred and fifty blood samples were collected from experimentally infected chickens for detection of *M. gallisepticum* antibodies in serum by different serological test. In the present investigation, the trials for reisolation of Mycoplasma gallisepticum from the respiratory organs of infected chickens showed that the highest recovery rate was from trachea (36%) followed by the air sacs (24.7%)

and finally the lungs (18%). It was found necessary to employ rapid, easy and accurate means of serodiagnosis and overcoming mycoplasma infection in farms. Serum plate agglutination (SPA) is most commonly used for screening purposes at age 1 day – 2 weeks. While the haemagglutination inhibition tests (HI) is most commonly used for confirmation of (MG) infection. SPA is rapid and sensitive assay, but however non specific agglutination reactions occurred is SPA. The application of Enzyme Linked Immunosorbant Assay (ELISA) as a diagnostic technique to serologically monitor anti MG antibodies when the infected birds at the age more than two week.

Key words: Serological tests, avian mycoplasma

INTRODUCTION

Mycoplasma in poultry has been recognized as being a respiratory problem, moreover, other economic losses due to poor growth, down grading of carcasses, low food conversion and lowering of egg production increased mortality have been reported (Jordan, 1979 and Oslan, 1984).

The spread of this disease in poultry farm takes place either vertically through infected egg as well as laterally such as by contact due to unhygienic measures in the farm.

Because of all hygienic and economic characters of the disease, it was found that necessary by poultry keepers to employ rapid, easy and accurate means of diagnosis and overcoming mycoplasma infection in their farms.

For instance, serum plate agglutination (SPA), haemagglutination inhibition (HI) and finally Enzyme Linked Immunosorbant Assay (ELISA) test have been recommended by many investigators to fulfill this purpose (Boyer *et al.*, 1960; Ansari *et al.*, 1983; Patten *et al.*, 1984; Talkington *et al.*, 1985 and Avakian and Kleven, 1990).

Each test has its own advantage and on the other hand disadvantage when used.

Therefore the aim of this work was to:

1- Compare the efficacy of different serological tests, serum plate agglutination test, (SPA); haemagglutination inhibition (HI) and finally Enzyme Linked Immunosorbant Assay (ELISA) in the detection of diseased birds with mycoplasmosis.

2- Detect the efficacy of each serological test as compared with the frequency of isolation of mycoplasma organisms in each case.

MATERIAL and METHODS

Mycoplasma strain:

Mycoplasma gallisepticum (S_6) strain and standard antisera are kindly supplied by Department of Mycoplasma Animal Health Research Institute, Dokki ,Giza.

I - Samples:

1- One hundred and fifty serum, Trachea, lung and airsacs samples were collected from chickens showing respiratory manifestation of different ages (one day old until two months) and different breeds from different localities (Cairo, Giza and Kaliobia governorates). Trachea, lung and airsacs samples were examined for mycoplasma isolation.

2- One hundred and fifty, Hubbard broiler chicks were obtained at one day old from Middle East poultry Company. All birds were examined by clinical and laboratory methods to be sure that they were free from Mycoplasma, at two weeks one hundred and twenty experimentally infected with virulent strain "S_6" M. gallisepticum. Serum samples were collected every week from experimentally infected chickens for detection of M. gallisepticum antibodies in serum.

II- Media

Media used for isolation PPLO medium (Adler *et al.*, 1958).

III- Biochemical identification

1- Glucose fermentation test:-

Glucose medium (Erno and Stipkovits , 1973).

2- Arginine deamination test:-

Arginine medium (Erno and Stipkovits , 1973)

IV- Serological diagnosis

1- Serum plate agglutination test (SPA). (Adler *et al.*, 1958).

2- Haemagglutination inhibition (HI). (Meszaros , 1964).

3- Enzyme Linked Immunosorbant Assay (ELISA) . This test was performed according to Higgins and Whithear (1985) using *M. gallisepticum* antibody kit (KIRKEGAARD and PERRY LABORATORIES (KPL).

V- Experimental infection

One hundred and fifty two week old chicks were divided into two groups (1) first group of one hundred and twenty infected with 0.1 ml of 10^8 CFU / ml of virulent strain S6 of *M. gallisepticum* at 15 days old chicks, thirty chicks were sacrified weekly till the end of the experiment for detection of antibodies against mycoplasma in sera. (2)

Second group, thirty chicks non infected kept as control negative, five chicks were sacrified weekly till the end of the experiment.

RESULTS

Table (1): Recovery rate of *M. gallisepticum* from different organs of infected chickens

Total samples	Recovery site	No. of positive	
		No.	%
150	Trachea	54	36
	Lung	27	18
	Air sac	37	24.7
Total		118	78.7

It was observed from table (1) that the highest rate of recovery of mycoplasma organisms was from the trachea followed by the air sac, then the lungs.

Table (2): Comparison between the different serological tests for detection of antibodies against *M. gallisepticum* in naturally infected chickens.

Age	Number of examined samples	Isolation		Serological test					
		No. of +ve	%	SPA		HI		ELISA	
				No	%	No	%	No	%
1 day old – 2 week	30	0	00	30	100	0	00	0	00
3rd week	30	5	16.7	30	100	10	33.3	18	60
4th week	30	13	43.3	30	100	17	56.7	25	83.3
5th week	30	23	76.7	30	100	30	100	30	100
6th week	30	25	83.3	30	100	30	100	30	100
Total	150	66	44	150	100	87	58.0	103	68.7

It is observed from table (2) that 1day -2 weeks old infected birds could be detected only using serum plate agglutination test (SPA) 100%. While other serological method i.e. HI and ELISA gave negative results at this period of age while at third week, all serological tests gave

different positive results with different percentages range from 33.3 % in HI test up to 60 % in ELISA, meanwhile, at fifth week, serological test gave 100% positive

Table (3): Comparison between the different serological tests for detection of antibodies against *M. gallisepticum* in experimentally infected chickens.

	Age	Number of examined birds	Re-isolation		Serological tests					
					SPA		HI		ELISA	
			+ve	%	NO	%	NO	%	NO	%
1st group infected with MG at 2 weeks	1ST w after infection 3rd w	30	20	66.7	30	100	0	0	0	0
	4th w	30	23	76.7	30	100	5	16.7	8	26.7
	5th w	30	28	93.3	30	100	18	60	25	83.3
	6th w	30	30	100	30	100	25	83.3	30	100
Control non infected		30	00	0	00	0	00	0	00	0
Total		150	101	67.3	120	80	48	32	63	42

It is observed from table (3) that the re-isolation percentage of M. gallisepticum in the 1st week post infection was 66.7%, increased gradually reaching 100% at the end of the experiment.

The results of antibody to *M. gallisepticum* infection detected by SPA gave positive results along the experiment.

The antibody response to *M. gallisepticum* detected by HI and ELISA were low in the 2nd week post infection then increased after that.

DISCUSSION

Respiratory mycoplasmosis in poultry is a major worldwide economic problem. Current methods to diagnose and manage *M. gallisepticum* infection include identification and elimination of infected birds (Brown *et al.*, 1991).

In the present investigation, the trials for reisolation of mycoplasma organisms from the respiratory organs of infected chickens showed that the highest recovery rate was from the trachea (36%) followed by the air sacs (24.7%) and the lungs (18%) as shown in table (1), this result agree with those reported by Yamamoto *et al.* (1992) Shaker (1995) and Dardeer (1997).

Serum plate agglutination (SPA) is most commonly used for screening purposes, while the haemagglutination inhibition test (HI) is most commonly used for confirmation of (MG) infections in chickens (Talkington *et al.*, 1985).

Enzyme Linked Immunosorbant Assay (ELISA) has been recently adopted for the diagnosis of MG antibodies in chickens (Opitz *et al.*, 1983).

It seems from our results that ELISA is applicable when the infected birds at age more than 2 weeks, this agree with the result of Dardeer , 1997).

The serum plate agglutination test is a rapid and sensitive assay, detecting immunoglobulin M (IgM) antibodies from few days after infections up to 72 days (Stipkovits , 1979).

However, non specific agglutination reactions occurred in SPA.The HI antibodies are in the IgG class, which normally appear from 2 weeks and remain detectable for several months after infection (Roberts, 1969 and Adler and Wiggins, 1973).

The application of ELISA as a diagnostic technique to serologically monitor anti *M. gallisepticum* antibodies in poultry holds great potential. At present, the SPA and HI tests are not ideally suitable to *M. gallisepticum* serodiagnosis, both assays lack characteristics that would allow a confident judgment of the *M. gallisepticum* status of a flock, thus a more sensitive and specific assay is needed, the ELİSA is an inherently sensitive test which could overcome at least some disadvantages of the other tests (Butler *et al.*, 1979 and Avakian *et al.*, 1988).

Our results of the experimental infection with *M. gallisepticum* proved that the SPA test can be used as an

easy and simple screening test at the age of 1 day – 2 weeks while the HI and ELISA tests can be used for confirmation, this results were similar to those of Talkington *et al.* (1985) and Kempf *et al.* (1994) who proved that the sensitivity of SPA was superior to that HI and ELISA in the ability to detect antibodies formed in early response to *M. gallisepticum* infection. However, it seems that both ELISA and HI tests had a higher degree of specificity.

REFERENCES

Adler, H.E.; Fabricant, J.; Yamamoto, R. and Berg, J. (1958): Symptoms on chronic respiratory disease of poultry. I. Isolation and Identification of pleuropneumonia like organisms of avian origin. Am. J. Vet. Res., 19: 440-447.

Adler, H.E. and Wiggins, A.D. (1973): Interpretation of serologic test for *M. gallisepticum*. World Poult. Sci.J., 29 : 345-353.

Ansari, A.A; Taylor, R.F. and Chang, T.S. (1983): Application of enzyme linked immunosorbant assay for detecting antibody to *M. gallisepticum* infections in poultry. Avian Dis., 27: 21-35.

Avakian, A.P. and Kleven, S.H. (1990): The humoral immune response of chickens to *M. gallisepticum* and *M. synoviae* documented by immunoblotting. Vet. Microbiol, 24: 1-15.

Avakian, A.P.; Kleven, S.H. and Glisson, G.R. (1988): Evaluation of the specificity and sensitivity of two commercial ELISA kits, the SPA and the HI for antibodies formed in response to *M. gallisepticum*. Avian Dis., 32: 262-272.

Boyer, C.I.; Fabricant, J. and Brown, J.A. (1960): Non specific plate agglutination reactions with pplo antigens. Avian Dis., 4: 546-547.

Brown, M.B.; Stolt, M.L.; Scassera, A.E. and Butcher, G.D. (1991): Detection of antibodies to *M. gallisepticum* in egg yolk versus serum samples. J. Clini. Microbiol. , 29 (12): 2901-2903.

Butler, J.E.; Feldbush, T.L.; Givern- MC, P. L. and Stewart, N. (1979): The enzyme linked immunosorbant assay (ELISA): A measure of antibody concentration Immunochemistry. 15: 131-136.

Dardeer, A.M. (1997): The efficacy of different modern techniques in the identification and serodiagnosis of Avian Mycoplasmosis .Ph. D. Thesis. Fac. Vet. Med. Cairo University.

Erno, H. and Stipkovits, L. (1973): Bovine mycoplasmas cultural and biochemical studies. Acta. Vet. Scand., 14 : 450-463.

Higgins, P.A. and Whithear, K.G. (1985): Detection and differentiation of *M. gallisepticum* and *M. synoviae* antibodies in chicken serum using ELISA. Avian Dis., 30 (1): 160-168.

Jordan, F.T.W. (1979): Avian mycoplasmas . The mycoplasmas. Vol. 11: 1-40.

Kempf , I.; Gesbert , M. ; Beenjean , G. and Stipkovits , L. (1994): Evaluation of two commercial enzyme linked immunosorbant assay kits for the detection of *M. gallisepticum* antibodies .Avian . Path., 23 (2) : 329-338.

Meszaros, J. (1964): Specificity and value of serological tests in the control of mycoplsmosis . Magy. Ao. Lapja, 19: 227-231.

Opitz, H. M. Duplessis, J.B. and Cyr , M.J. (1983): Indirect micro-enzyme linked immunosorbant assay for the detection of antibodies to *M. gallisepticum* and *M.synoviae*. Avian Dis., 27 :773-786.

Oslan, H.M. (1984): M. synoviae infection. In Hofstad , M.S.; H.J. Barnes ; B.W. Calnek ; W.M. Reid and H.W. Yoder (Editors), Diseases of poultry , 8th Edition . Iowa State University Press, Ames, I.A., pp. 212-220.

Patten, B.E.; Higgins P.A. and Whithear, K.G. (1984): A urease ELISA for the detection of mycoplasma in poultry. Aust. Vet. J., 61 (5): 151-155.

Roberts, D.H.(1969): Serological response produced in chickens by 3 strains of *M. gallisepticum* . Appl. Bacterial., 32 : 395-401.

Shaker, M. M. (1995): Microbiological studies on mycoplasma infection in poultry, Ph. D. Thesis, Fac. Vet. Med., Cairo University.

Stipkovits, L. (1979): The pathogenicity of avian mycoplasmas . Zbl. Bakt. Hyg., I. Abt., Orig., A. 245 : 171-183.

Talkington, F.D.; Kleven, S.H. and Brown, S.H. (1985): An ELISA for the detection of antibodies to M. gallisepticum experimentally infected chickens. Avian Dis., 29: 53-70.

Yamamoto, R.; Zaini, M.Z.; Tan, L.J. and Kuniyasu, C. (1992): Bacteriological and serological survey of avian mycoplasmosis in peninsula Malaysia JAR. Q. Japan Agricultut. Res. , 25 (4) : 278-282.