Dept. of Mycoplasma
Animal Health Research Institute, Dokki, Cairo
Director Prof. Dr. A.F. Abdel Ghawaad

THE APPLICATION OF IMMUNOPEROXIDASE TEST FOR THE DIAGNOSIS OF AVIAN MYCOPLASMA

(With 2 Tables)

By

LAILA M. EL-SHABINY; MANAL ABO EL-MAKAREM*
and NAGAT SALEH

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إستخدام إختبار البيروكسيديز المناعى فى تشخيص ميكوبلازما الطيور

ليلى الشعبينى ، منال أبو المكارم
نجاة صالح

من الدجاج والبيض المختار والمجمع عشوائياً من محافظة الشرقيه تم فحص عدد ٤٠ عينة نسيج و ٤٠ عينة مصل و ٤٠ عينة مح بيض و١٠ عينات من السائل السينوفى وكذلك عدد ١٠ عينات من غسول القصبه الهوائيه . وذلك لوجود الميكوبلازما والأجسام المضاده للميكوبلازما بإستعمال إختبار البروكسيديز المناعى ، وقد عقدت مقارنه بين نتائج الفحص بالبيروكسيديز المناعى ونتائج طريقة الزرع وإختبار التلازن وإختبار مانع التجمع الدموي وقد وجد أن إختبار البيروكسيديز المناعى أكثر دقه وأكثر حساسيه .

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*: Buffalo Disease Research Department,
Animal Health Research Institute, Dokki, Cairo.

SUMMARY

Forty tissue samples, 40 chicken sera; 40 egg yolk samples, 10 synovial fluid samples and 10 tracheal washings were collected from randomly selected chickens and eggs from Sharkia Governorate. These samples were submitted to examination with indirect immunoperoxidase (IP) test for the presence of Mycoplasma antigens or antibodies. A comparison was held between the results obtained and those using the isolation method, haemagglutination inhibition test (HI) and slide agglutination (SA); the (IP) was found to be more sensitive and accurate.

INTRODUCTION

Mycoplasma infection is one of the important endemic poultry diseases in Egypt causing economic loss due to condemnation rate and reduction of egg production and high medication cost, for these reasons an accurate, rapid and simple method of diagnosis is required.

Immunoperoxidase test was previously used by some authors allover the world for the diagnosis of mycoplasma such as *BRUGMANN et al. (1977); BRUGMANN and KELLER (1977); POLAK-VOGELZANG et al. (1978); HILL (1978) and IMADA et al. (1979).*

In Egypt, *EL-SHABINY et al. (1992)* started the first trial for the diagnosis of bovine mycoplasma followed by another study by *EL-SHABINY et al. (1993)* for the identification of bovine mycoplasma using filter paper discs.

MATERIAL and METHODS

Samples :

1- Tissue samples: tissue samlples (from lung, air sac and trachea) from 40 chickens with respiratory signs from a flock at El-Sharkia Governorate in Egypt.
2- Serum sample: 40 serum samples were obtained from the same birds.
3- Egg samples: 40 yolk samples (local eggs).
4- Respiratory secretions: 10 tracheal washings.
5- Synovial fluids: 10 synovial fluids.

Tissue samples were cultivated on Frey's medium *(FREY et al., 1968)* according to the method of *HAYFLICK (1965).* Biochemical characterization was made as described by *SABRY (1968)*, genus determination according to *ERNO and STIPKOVITS (1973)*, and identification of the isolates was according to

CLYDE (1964), using growth inhibition test. Serum samples and chloroform extracted yolk samples were submitted to examination by [SA] *(ADLER et al., 1958)*, [HI] *(MESZAROS, 1964)* and indirect [IP] *(BENCINA and BRADBURY, 1991)*. Respiratory secretions and synovial fluids were examined by culture method and IP test.

Mycoplasma gallisepticum type cultures and antisera were obtained from "National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, 20014, U.S.A.". *M. gallisepticum* and *M. SYNOVIAE* stained antigens were those of Intervet, Boxmeer, Holland.

IP test: Known *M.gallisepticum* culture strain S6 and *M.synoviae* strain MVU or unknown isolates cultured on Frey's medium without phenol red and their colonies on agar plates served as target antigens, with a marker, 2 circles about 1/4 inch diameter at the bottom of the plates where there is a good growth marked and labeled 1,2. Discs saturated with rabbit antisera were placed on appropriate circles or unknown chicken sera or egg yolk extract or tracheal washings. Plates were inclubated at 37°C for 2 hours then discs were removed. Discs saturated with HRP (horse radish peroxidase), rabbit anti-chicken IgG diluted 1/50 in TBS with 1% B.S.A. were applied where the first discs were placed. Plates were incubated at 37°C for 2 hours, discs were removed then plates were washed once with wash solution, plates were covered with developer solution, the reaction was left until a dark purple colour developed on control colonies then washed briefly. Wash solution: 100 ml TBS, 2 ml horse serum, 1 drop tween 20. Developer solution: 20 ml ice cold methanol and 60 mg 4,chloro-1-naphthol mixed before use with 100 ml TBS and 60 ul H_2O_2.

Positive samples were examined with homologous and heterologous cultures or antisera of *M. gallisepticum* and *M. synoviae*.

RESULTS

Results presented in Table (1) showed that, after using SA test, 12 out of 40 serum samples were positive for *M. gallisepticum* and 16 out of 40 were *M. synoviae* positive. Using the HI test the number of positives decreased. IP test revealed 14 *M.gallisepticum* positive serum samples and 16 *M. synoviae* positive samples from 40; i.e. IP test showed the highest number of positive while the HI and the culture methods gave lower positive numbers.

Regarding the egg yolk samples, also IP test was as sensitive as the culture method and the HI method showing 16 *M.gallisepticum* and 12 *M. synoviae* positive samples out of 40, while SA test showed the lowest number of positive samples.

It was clear from the results of Table (2) that, IP test can be used for detecting antibodies in the respiratory secretions and synovial fluids although the isolation method could detect more cases. Regarding tissue samples, it was found that IP test gave the same positive number as the culture method.

It was found that there was no cross reactions using homologous and heterologous cultures and/or antisera indicating the specificity of IP test.

Table 1: Examination of tissue, serum and egg yolk for Mycoplasma infeciton

Sample	Serological examination						Culture method isolation	
	SA		HI		IP			
	Mg	Ms	Mg	Ms	Mg	Ms	Mg	Ms
Tissue samples	-	-	-	-	10/40	14/40	10/40	14/40
Serum samples	12/40	16/40	10/40	14/40	14/40	16/40	-	-
Yolk samples	8/40	4/40	16/40	12/40	16/40	12/40	16/40	12/40

N.B. 1: IP for serum samples and egg yolk samples was done at 1/30 dilution.

2: The numerator indicates the number of positives and the denominator the number examined.

3: Mg = *Mycoplasma gallisepticum.*
Ms = *Mycoplasma synoviae.*

Table 2: Examination of respiratory secretions and synovial fluids for Mycoplasma infection.

Sample examined	IP		Isolation	
	Mg	Ms	Mg	Ms
Respiratory secretion	3/10	2/10	4/10	3/10
Synovial fluids	2/10	3/10	3/10	4/10

N.B. 1: The numerator indicates the number of positives, the denominator the number examined.

2: Mg = *Mycoplasma gallisepticum.*
Ms = *Mycoplasma synoviae.*

DISCUSSION

From the results of Table (1), it is clear that regarding serum samples, HI showed lower number of Mycoplasma positives compared with SA because it is less sensitive although it is

more specific especially for detecting Mycoplasma infection in egg yolk in which the main antibodies are of the IgG type while SA depends on IgM antibodies *(MOHAMMED et al., 1986)*. Regarding tissue samples, it was clear that IP test was as sensitive as the culture method.

The results of the present study emphasizes the importance of IP test for the detection of antigens as well as antibodies against *M.gallisepticum* and *M.synoviae* in chicken sera, respiratory secretions, synovial fluids and egg yolk. it was proven to be simple and accurate. Using IP test, no cross reaction was found between *M.gallisepticum and M.synoviae.* It was more specific than SA test. Our results are in agreement with those of *IMADA et al (1982 & 1987)* who concluded that IP was specific and sensitive. *BENCINA and BRADBURY (1991)* proved the potential of IP for detecting *M. gallisepticum and M. Synoviae* antibodies in serum, egg yolk and respiratory secretions and confirmed that it was more sensitive than the culture method. Also, they referred to a very recent preliminary study indicating that IP test may have an advantage of being specific than ELISA because broth antigen of ELISA has more internal rather than cell membrane antigens and this may lead to cross reaction between some species of Mycoplasma sharing certain antigens, while the agar colonies were superior and showed no cross reactions. *IMADA et al (1987)* reported that immunobinding test for Mycoplasma cells in broth culture blotted on microcellulose membrane leads to weak staining and enhancing cross reaction besides IP can be more readily applied than immunobinding.

REFERENCES

Adler, H.E.; Fabricant, J.; Yamanmoto, R. and Berry, J. (1958): Isolation and identification of pleuropneumonia like organism of avian origin. Am. J. Vet. Res., 19: 44-47.

Bencina, D. and Bradbury, J.M. (1991): Indirect immunoperoxidase assay for the detection of antibody in chicken mycoplasma infection. Av. Path. 20: 113-124.

Bruggann, S. and Keller, H. (1977): Quantitative detection of antibodies to *M. suipneumoniae* in pigs sera by an enzyme-linked immuno sorbent assay. Vet. Rec. 101: 109-111.

Brugmann, S.; Engberg, B and Ehrensperger, F. (1977): Demonstration of *M. suipneumoniae* in pig lungs by the enzyme linked immuno peroxidase technique. Vet. Rec., 101, 137.

Clyde, W.A. (1964): Mycoplasma species identification based upon growth inhibition by specific antisera. J. Immunol., 92(6): 958-965.

El-Shabiny, L.M.; El-Tarabili, M.M. and El-Sayed, R.F. (1992): The application of immunoperoxidase test for the diagnosis of Mycoplasma infection in buffaloes. Vet. Med. J., Giza, 40: 37-41.

El-Shabiny, L.M.; Shin, S.J. and McDonough, P.L. (1993): A simple immunoperoxidase test for identification of bovine mycoplasma. J. Egypt. Vet. Ass., 53: 31-35.

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

Frey, M.L.; Hanson, P. and Anderson, D.P. (1968): A medium for the isolation of avian mycoplasmas. Am. J. Vet. Res., 29: 2163-2171.

Hayflick, L. (1965): Tissue culture and mycoplasmas. Tex. Resp. Biol. Med. 23: 285-303.

Hill, A.C. (1978): Demonstration of mycoplasma in tissue by immunoperoxidase techique. J. Inf. Dis., 137: 152-154.

Imada, Y.; Nomomura, I. and Furuta, K. (1982): Indirect immunoperoxidase technique for the assay of antibodies against *M. gallisepticum and M. synoviae.* National Institute of Animal Health, 22: 16-22.

Imada, Y.; Uchida, I. and Hashimoto, K. (1987): Rapid identification of mycoplasma by indirect immunoperoxidase test using small square filter paper. J. Clin. Microbiol., 25: 17-21.

Imada, Y.; Nomomura, I.; Hayashi, S. and Tsurubuchi, K. (1979): Immunoperoxidase technique for identification of *M. gallisepticum and M. synoviae.* National Institute of Animal Health, 19: 40-46.

Meszaros, J. (1964): Specificity and value of serological tests in the control of mycoplasmosis. Nagy, AO. Lapja 19: 227-231.

Mohammed, H.O.; Yamamoto, R.; Carpenter, T.E. and Ortmayer, H.B. (1986): Comparison of egg yolk and serum for the detection of *Mycoplasma gallisepticum and M. synoviae* antibodies by enzyme linked immunosorbent assay. Avian Dis., 30: 398-408.

Polak-Vogelzang, A.A.; Hagenaars, R. and Nage, S. (1978): Evaluation of an indirect immunoperoxidase test for identification of Acholeplasma and Mycoplasma. J. Gen. Microbiol., 106: 241-249.

Sabry, M.Z. (1968): Characterization and classification of avian mycoplasma. Ph.D. Thesis, Cornell Univ., U.S.A.