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APPLICATION OF SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) FOR IDENTIFICATION OF MYCOPLASMA INFECTION IN TURKEYS WITH SPECIAL REFERENCE TO TREATMENT

S.I. EISSA*; M.A. DARDEER* and M.A. ABO-NORAG**

*Department of Mycoplasma, Animal Health Research Institute, Dokki.
**Department of Biochemistry, Animal Health Research Institute, Dokki.

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SUMMARY

A total of sixty fluid exudate samples aspirated from the infra-orbital sinus of turkey poults suffered from respiratory manifestations were examined for mycoplasma infection. Mycoplasma synoviae could be isolated from 13.33% of samples, while *M. gallisepticum* was recovered from only 6.66% of the examined samples.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of some differences in the protein makeup of M. synoviae field isolates compared with that of the standard reference strain, which indicates strain variability. However, *M. gallisepticum* reference strain and field isolates revealed largely similar electrophoretic patterns of their cell proteins.

The efficacy of enrofloxacin, lincospectin and tylosin as antimycoplasmal drugs for the treatment of turkey poults naturally infected with *M. synoviae* and *M. gallisepticum* was studied. The minimum inhibitory concentration (MIC) of enrofloxacin ranged from 0.006 to 0.012 (µg/ml, lincospectin was 0.024 (µg/ml, while tylosin had the least activity (0.024-0.048 (g/ml). Mycoplasma could be re-isolated from only one of 20 turkey poults treated with enrofloxacin, 3 out of 20 birds treated with lincospectin and 5 out of 20 birds treated with tylosin.

INTRODUCTION

Mycoplasma synoviae (MS) infection most frequently occurs as a subclinical upper respiratory disease that may become systemic and result in infectious synovitis, that is an acute or chronic

infectious disease of chickens and turkeys involving primary the synovial membrane of joints and tendon sheaths (Kleven et al., 1991). Horizontal transmission occurs readily by direct contact via the respiratory tract, and vertical transmission from infected breeder hens to their progeny has played a major role in the initiation of M. synoviae outbreaks (Kleven et al., 1991).

Infection of layer flocks with field strains of M. gallisepticum (MG) may cause significant egg production drop (Glisson and Kleven, 1984; Cummings and Kleven, 1986). Also, infection of broiler flocks cause decreased body weight and low feed conversion (Yoder, 1984). Recently, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has indicated unnoticed heterogeneity among strains of M. gallisepticum and M. synoviae (Avakian et al., 1991; Chin et al., 1991; Gibbs et al., 1994).

The minimum inhibitory concentration (MIC) of enrofloxacin was between 0.012-0.024 (µg/ml for of M. gallisepticum and M. synoviae (Eissa, 1996), while that of tylosin was between 0.016-0.064 (µg/ml (Bradbury et al., 1994). Enrofloxacin proved to be effective for treatment of broiler chickens experimentally infected with of M. gallisepticum (Abd El-Aziz et al., 1996).

Objectives of the present study were:

To evaluate SDS-PAGE for identification of M. gallisepticum and M. synoviae infection in turkeys.

To compare the efficacy of enrofloxacin, lincospectin and tylosin for treatment of mycoplasma infection in turkeys.

MATERIAL AND METHODS

Samples:

A total of sixty fluid exudate samples were aspirated from the infra-orbital sinus of turkey poults aged from 3-5 months. All birds were suffered from respiratory manifestation with swelling of the infra-orbital sinus.

Media:

Liquid and solid media were used for the isolatio and propagation of mycoplasma were prepared described by Frey et al. (1968). Biochemical cha acterization of the isolated purified strains wa carried out as described by Erno and Stipkovi (1973). The isolates were identified by growth i hibition test according to Clyde (1964).

Sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE):

Proteins of mycoplasma strains were separated SDS-PAGE using a discontinuous buffer sys as described by Laemmli (1970). Briefly, wh cell samples of each protein and molecular w standards were diluted in sample buffer, h for 5 minutes, and electrophoresed in 1 mm

10% gel under constant current. Proteins were stained with 1% Coomassie brilliant blue, destained, then photographed and analyzed by densitometer, (Zeineh, USA).

Reference Mycoplasma strains : M. gallisepticum and M. synoviae reference strains were obtained from Dr. S.A.EL-Shater, Department of Mycoplasma, Animal Health Research Institute.

Drugs:

Enrofloxacin (Spectrama-Vet ®) obtained in the form of injectable solution 10% obtained form AMOUN Pharmaceutical Industries Company, Cairo Egypt.

Lincomycin hydrochloride and Spectinomycin sulphate (Linco-spectin®) obtained in the form of injectable solution, each ml contains 50 mg lncomycin hydrochloride , 100 mg spectinomycin sulfate. It was obtained from Pharmacia, Upjhon, USA.

Tylosin tartrate (Tylan 50®) obtained in the form of injectable solution of tylosin base 50% produced by Lilly, Liverpool, England.

Titration of mycoplasmas:

The number of colour changing units (CCU) was determined by the method described by Taylor-Robinson (1983). A titre of 10^3-10^4 CCU/0.2 ml was required for the test proper (Senterift, 1983).

Determination of minimal inhibitory concentration (MIC):

The test was performed in the duplicate as described by Senterfit (1983). The antimicrobials were tested in serial two fold dilutions at concentration ranging from 6 to 0.006 (g/ml.

Treatment:

Sixty diseased turkey poults aged 3-5 months were isolated from a turkey flock in a private farm. Birds were divided into three groups A, B and C (20 for each). At the first day, the three groups were treated intrasinusly with enrofloxacin (10 mg/kg b.wt.) for group A, lincopsectin (0.2 ml/kg b.wt.) for group B and tylosin (10 mg/kg b.wt.) for group C. At the 2nd and 3rd day, birds in each group were injected intramuscularly with the corresponding dose of drug. Clinical symptoms of all birds in each group were observed and recorded. At the 7th day, exudate samples from the infra-orbital sinus were aspirated for re-isolation of mycoplasma.

RESULTS

Investigation of 60 exudate samples aspirated from the infra-orbital sinus of diseased turkey poults was carried out for detection of mycoplasma infection. Out of 60 samples, 12 mycoplasma strains were isolated. Eight isolates were identified as M. synoviae, while 4 isolates were M. gallisepticum (Table, 1).

Table (1): Isolation and identification of mycoplasma from turkey poults.

Site	No. Examined	No. Positive	Digitonin	Biochemical characterization				Serological identification
				G	A	Fl&S	No. of isolates	
Sinus	60	12	+	+	-	+	8	*M. synoviae*
			+	+	-	-	4	*M. gallisepticum*

G = Glucose A= Arginine

F&S = Film and spot

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of M. gallisepticum reference strain and field isolates revealed largely similar electrophoretic pattern of cell proteins (From 85 to 18 kilodaltons).

The protein profile of M. synoviae field isolates was compared with the standard reference strain, some differences were detected in the upper portion of the gel, indicating strain variability (Fig., 1).

The in vitro activities of enrofloxacin, lincospectin and tylosin against 8 isolates of Mycoplasma synoviae and 4 Mycoplasma gallisepticum isolates, as determined by the micro-broth technique are shown in table (2). Of the three antimicriobials, enrofloxacin had the highest activity (0.006-0.012 (g/ml), followed by lincopsectin (0.024 (g/ml), while tylosin was the least (0.024-0.048 (g/ml).

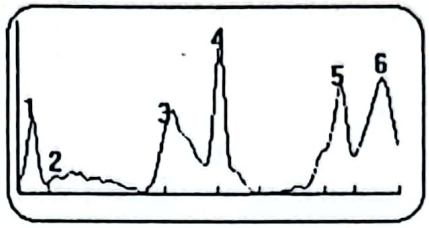
Our results proved that enrofloxacin has a superior efficacy than lincospectin and tylosin in treatment of mycoplasma infection in turkey poults. Mycoplasma could be re-isolated from only one bird of 20 treated with enrofloxacin, 3 birds of 20 treated with lincospectin and 5 birds of 20 treated with tylosin.

Fig. (1): Electrophoretic pattern of M. synoviae and M. gallisepticum isolated from turkeys.
1: Low Molecular mass standard.
2: Mycoplasma gallisepticum (Reference strain).
3: Mycoplasma synoviae (Reference strain).
4-7: Mycoplasma synoviae (Field isolates).
8-11: Mycoplasma gallisepticum (Field strains).
12-15: Mycoplasma synoviae (Field isolates).

Table (2): Electrophoretic Pattern of Mycoplasma gallisepticum (PG31 Reference Strain)

Low Molecular Weight Standard

PK	MW	Ht	Area	%Amt
1	97400	1142	8446	7.81
2	66200	281	13440	12.43
3	45000	1081	25761	23.82
4	31000	2280	14067	13.00
5	21500	1686	17308	16.00
6	14400	1880	29145	26.94



Mycoplasma gallisepticum (PG31)

PK	MW	Ht	Area	%Amt
1	105347	464	32176	28.67
2	93894	269	6730	6.00
3	85306	1211	10867	9.68
4	78251	938	4689	4.18
5	71094	111	492	0.44
6	66478	268	10154	9.05
7	51805	369	3662	3.26
8	45295	145	6658	5.93
9	35298	376	11475	10.23
10	29417	696	4420	3.94
11	26219	90	669	0.60
12	22706	124	1592	1.42
13	21331	260	7783	6.94
14	18211	569	10848	9.67

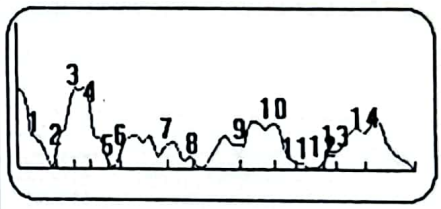
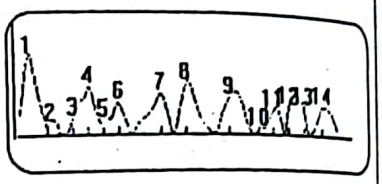


Table (3): Electrophoretic Pattern of Mycoplasma synoviae and M.gallisepticum.

Mycoplasma gallisepticum field isolate

PK	MW	Ht	Area	%Amt
1	109467	1429	16841	18.87
2	96635	171	1798	2.01
3	85306	266	4973	5.57
4	78251	841	4616	5.17
5	71780	247	3261	3.65
6	65843	573	7933	8.89
7	51805	726	6024	6.75
8	44863	912	10693	11.98
9	34627	655	7972	8.93
10	28858	91	1827	2.05
11	26219	433	3348	3.75
12	23594	511	6504	7.29
13	21231	491	2930	3.28
14	18563	523	10517	11.79



Mycoplasma synoviae reference strain

PK	MW	Ht	Area	%Amt
1	110522	247	13337	19.92
2	94799	5	83	0.07
3	89497	40	542	0.46
4	83685	160	3349	2.86
5	76032	832	12094	10.32
6	67118	496	9006	7.69
7	54350	836	6747	5.76
8	46618	108	4406	3.76
9	41950	567	5241	4.47
10	36679	975	22599	19.29
11	29987	372	0	0.00
12	24517	1063	15122	12.91
13	22062	942	4873	4.16
14	19853	240	2873	2.45
15	16705	391	6863	5.86

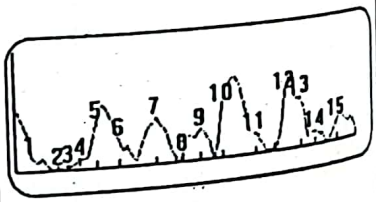
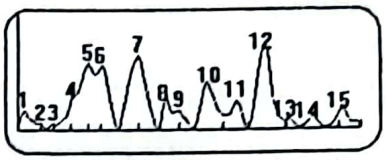


Table (4):Electrophoretic Pattern of Mycoplasma synoviae isolated from turkeys.

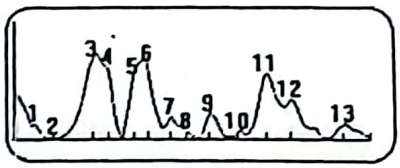
Mycoplasma synoviae field isolate

PK	MW	Ht	Area	%Amt
1	109467	370	17711	15.70
2	98506	102	445	0.93
3	91230	98	2123	1.88
4	82095	564	9559	8.48
5	73875	1327	9688	8.59
6	68418	1270	18176	16.12
7	54350	1498	9416	8.35
8	46618	490	4799	4.26
9	42354	358	1694	1.50
10	35298	857	9601	8.51
11	29987	544	5060	4.49
12	25721	1507	18157	16.10
13	22274	148	148	0.13
14	19475	118	3316	2.94
15	16076	302	2889	2.56



Mycoplasma synoviae field isolate

PK	MW	Ht	Area	%Amt
1	106362	283	19111	16.33
2	94799	2	19674	9.12
3	74587	1555	15797	13.50
4	67765	1380	9537	8.15
5	58124	1210	13023	11.13
6	53317	1441	8356	7.14
7	46618	410	2851	2.44
8	42354	126	1509	1.29
9	37032	452	3772	3.22
10	31160	113	7260	6.21
11	26219	1273	13350	11.41
12	22706	753	8768	7.49
13	16545	279	2994	2.56



DISCUSSION

In the present study, M. synoviae could be isolated from 13.33% of the examined samples, while the rate of isolation of M. gallisepticum was 6.66%. El-Ebeedy (1977) reported that most of the turkey flocks in Hungary were infected with M. meleagridis, while only few flocks were infected with M. gallisepticum. No M. synoviae infection was observed. Abd El-Rahman (1995) concluded that M. gallisepticum infection was higher (53.4%) than that of M. synoviae (9.3%) in the examined turkey flocks.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profiles of M. gallisepticum reference strain and the field isolates revealed largely similar electrophoretic patterns of cell proteins (Fig., 1). Avakian et al. (1992) proved that M. gallisepticum isolated from turkeys were similar to that of M. gallisepticum reference strain in species specific regions of their electrophoretic profiles.

The electrophoretic patterns of M. synoviae field isolates showed some differences when compared with that of the standard reference strain, indicating strain variability (Fig., 1). Heterogeneity among M. synoviae strains may be explained by the evidence that mycoplasma have higher mutation rates than other bacteria, which has been postulated to account for the marked genotypic and phenotypic diversity of the organisms constituting the class Mollicutes (Ley and Avakian, 1992).

Results from our micro-broth tests have shown that enrofloxacin is active in vitro against M. gallisepticum and M. synoviae (0.006-0.012 (µg/ml), followed by lincopsectin (0.024 (µg/ml) and tylosin was the least (0.024-0.048 (µg/ml). Our results were in agreement with those obtained by Bradbury et al. (1994) and Eissa (1996) who found that M. gallisepticum and M. synoviae were sensitive to enrofloxacin, while tylosin was less effective.

Our results revealed that enrofloxacin has a superior efficacy than lincospectin and tylosin in treatment of mycoplasma infection in turkeys. The antimycoplasmal effect of enrofloxacin was determined by Berg (1988) and Chen et al. (1994). This effect may be due to high concentration of enrofloxacin achieved in saliva, nasal secretion, nasal mucosa and bronchial secretion (Neu, 1988). In addition, Carlier et al. (1990) reported that enrofloxacin gain entry into phagocytic cells and remain microbiologically active inside the cell against bacterial pathogen. Moreover, the efficacy of enrofloxacin proved by low MIC (0.006 µg/ml - 0.0012µg/ml) and re-isolation of mycoplasma from just 5% of treated turkeys.

Combination of lincomycin with spectinomycin enhanced the activity against mycoplasma infection (Burrows, 1980). This activity could be explained due to high lipid solubility and

distribution of lincomycin in the body as well as its tissue concentration exceed serum concentration by several times. Moreover, spectinomycin is a specific antimycoplasmal drug. Therefore, mycoplasma could be re-isolated from 3 birds of twenty treated by lincospectin.

Tylsoin tartarate is a bacteriostatic has a relatively high MIC (0.048 µg/ml) in comparison to enrofloxacin (0.012 µg/ml) and lincospectin (0.024 µg/ml). Tylosin was not as effective as enrofloxacin or lincospectin in treatment of mycoplasmosis in turkeys. Mycolpasma could be re-isolated from 25% of treated birds. This result was in agreement with Hannan (1982) who reported that tylosin was not effective in controlling an experimental mixed mycopalsma and bacterial pneumonia.

REFERENCES

Abd El-Aziz, M.I.; El-Banna, H.A. and Eissa, S.I. (1996): "Efficacy and residual pattern of some fluoroquinolones in poultry." J. Egypt. Vet. Med. Ass., 56 (4): 547-562.

Abd El-Rahman, F.I. (1995): "Incidence of mycoplasmal infections in turkeys." Vet. Med. J., Giza, 43 (2): 201-206.

Avakian, A.P.; Kleven, S.H. and Ley, D.H. (1991): "Comparison of Mycoplasma gallisepticum strains and identification of immunogenic integral membrane proteins with triton X114 by immunoblotting." Vet. Micrbiol., 29: 319-328.

Avakian, A.P.; Ley, D.H. and McBride, M.A. (1992): "Humeral immune response of turkeys to strain S6 and a variant Mycoplasma gallisepticum studied by immunoblotting."Avian Dis., 36: 69-77.

Berg, J.N. (1988): "Clinical indication of enrofloxacin in domestic animals and poultry." Proceeding of the Western Veterinary Conference, Las Vegas.

Bradbury, J.M.; Christine, A.Y. and Giles, C.J. (1994): "In vitro evaluation of various antimicrobials against Mycoplasma gallisepticum and Mycoplasma synoviae by the micro-broth method, and comparison with a commercially prepared test system." Avian Pathol., 23: 105-115.

Burrows, G.E. (1980): "Pharmacotherapeutics of macrolides, lincomycin and spectinomycin." J. Am. Vet. Med. Ass., 176: 1072.

Carlier, M.B.; Scorneaus, B.; Zenebergh, A.; Desnoltes, J.F. and Tulkens, P.M. (1990): "Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages." J. Antimicrob. Chemother., 26 (B): 27-39.

Chen, Z.; Fung, K.F.; Fang, B. and Song, Y. (1994): "Antimicrobial and pharmacokinetic studies of fluoroquinolones in chickens." Proceeding of the 6th Congress of the European Association for Veterinary Pharmacology and Toxicology., Edinburgh, Scotland, pp. 209-210.

Chin, R.P.; Meteyer, C.U.; Yamamoto, R.; Shivaprasad, H.L. and Klein, P.N. (1991): "Isolation of Mycoplasma synoviae from the brains of commercial meat turkeys with Meningeal vasculitis." Avian Dis., 35: 631-637.

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

Cummings, I.S. and Kleven, S.H. (1986): "Evaluation of protection against Mycoplasma gallisepticum infection in chickens vaccinated with the F strain of Mycoplasma gallisepticum." Avian Dis., 30: 169-171.

Eissa, S.I. (1996): Minimal inhibitory Concentrations of quinolones group to some avian mycoplasmas.I Vet. Med. J. Giza, 44 (3): 563-568.

El-Ebeedy, A.A. (1977): "Studies on mycoplasma infection of turkeys, geese and ducks." Candidate D. Thesis, Hungarian Academy of Science, Budapest.

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

Frey, M.C.; Hanson, R.P. and Anderson, D.P. (1968): " A medium for isolation of avian mycoplasma." Am. J. Vet. Res., 29: 2164-2171.

Gibbs, P.S.; Kleven, S.H. and Joackwood, M.W. (1994): "Analysis and characterization of Mycoplasma gallisepticum isolates from Pennsybvania." Avian Dis., 38: 475-482.

Glisson, J.R. and Kleven, S.H. (1984): "Mycoplasma gallisepticum vaccination effects of egg transmission and egg production." Avian Dis., 28: 406-415.

Hannan, P.C.T. (1982): "Tylosin tartrate and tiamulin effects on experimental piglet pneumonia induced with pneumonic pig lung homogenate containing mycoplsma, bacteria and virus." Res. Vet. Sci., 33:76.

Kleven, S.H.; Rowland, G.N. and Olson, N.O. (1991): iMycoplasma synoviae infection. In: Diseases of poultry. 9th Ed., pp. 223-231, Calnek, B.W.; Barnes, H.J.; Beard, C.W.; Reid, W.M. and Yoder, Jr. H.W.; (eds), Iowa State University Press, Ames, Iowa.

Laemmli, U.K. (1970): Cleavage of structural proteins dur- ing the assembly of the head of bacteriophage T4. Na- ture, 227: 680-685.

Ley, D.H. and Avakian, A.P. (1992): An outbreak of Myco- plasma synoviae infection in North Carolina turkeys." Comparison of isolates by sodium dodecyl sulfate- polyacrylamide gel electrophoresis and restriction endo- nuclease analysis." Avian Dis., 36: 672-678.

Neu, H.C. (1988): "Quinolones: A new class of antimicrobi- al agents with wide potential uses." Med. Clin. North Am., 72: 623-636.

Senterfit, L.B. (1983): "Antibiotic sensitivity testing of my- coplasmas. In Methods in Mycoplasmology." Vol. II, pp. 397-401, Academic Press.

Taylor-Robinson, D. (1983): "Metabolism inhibition tests In Methods in Mycoplasmology." Vol. I, pp. 411-417 Academic Press.

Yoder, H.Jr. (1984): "Mycoplsma gallisepticum infection In Diseases of Poultry. 8th Ed., pp. 190-212, M.S. Hof- stad; H.J. Barnes; B.W. Calneck; W.M. Reid and H.W. Yodre Jr (eds), Iowa State University Press, Ames Iowa