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STUDIES ON BACTERIA ASSOCIATED WITH RESPIRATORY DISORDER IN POULTRY WITH SPECIAL REFERENCE TO PASTEURELLA MULTOCIDA

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ABSTRACT

Trials for isolation of Pasteurella multocida from lung samples of chickens suffering from Pneumonia were curried out. The identity of Pasteurella multocida was proved by mouse inoculation and by polymerase chain reaction (PCR). 120 samples with pneumonia were collected from 500 slaughtered chickens with history of respiratory disorders. All samples inoculated into blood agar supplemented with 10% sheep blood. The bacteriological examination was carried out on all samples and revealed that the incidence of P. multocida was 6 (5%). In addition, mouse inoculation test was carried out on P. multocida suspected isolates. Five (83.3%) of isolates were all positive and one (16.3%) isolate not lethal for mice. All P. multocida strains that were positive by culture were also detected to be positive by the PCR. Toxigenic P. multocida was not isolated from any of these specimens .

INTRODUCTION

Respiratory infection is the most serious disease affecting poultry and causes heavy economic losses in the poultry industry worldwide.

Avian respiratory diseases caused mainly by several micro organisms of the genus *Pasteurella* (*P. multocida,P. gallinarum, P. haemolytica* and *P. anatipestifer*),*Bordetella* (*B. avium*) and *Haemophilus (H. gallinarum)* are involved in respiratory disease complex ***(Hafez,2002).*** The Gram-negative bacterium *Pasteurella multocida* exhibits a broad host range including most mammals,P. *multocida* has been consistently found in the upper respiratory tract, birds and also humans ***(Adlam and Rutter, 1989; Quinn et al., 1994).*** Tracheitis,exudative pneumonia, pleuritis,air sacculitis, pericarditis, sinusitis characterize the infection ***(Canal et al., 2005).***

Detection of *P. multocida* as causative agent of avian cholera and respiratory manifestation is important in the overall control and elimination of this disease from poultry flocks. Even through methods used to isolate *P.multocida* from tissues, which are normally sterile sites ,are adequate for the clinical diagnosis of avian cholera and respiratory manifestation. Selective media ***(Morris, 1958 and Knight et al., 1983).***

Or mouse inoculation ***(Pedersen and Barfod 1981)*** method used to detect *P. multocida* mixed with other microorganisms have serious weakness. Results from studies using selective media have been disappointing ***(Morris,1958 and Moore et al.,1994).*** Some media will not support the growth of all possible isolates, whereas others are too insensitive. Mouse inoculation on the other hand, appear to be more sensitive than many selective media ***(Pedersen and Barfod 1981)*** and less strain dependant, i.e.most, if not all ,strains of *P.multocida* will affect mice ***(Collins, 1973 and Okarman, et al., 1979).*** The disadvantage of this methods are expensive and the use of mouse for each sample.

DNA-based methods allow the detection of organisms directly from clinical samples thus dramatically improving the sensitivity and decreasing the time required for bacterial identification ***(Townsend et al., 1998).***

Also relatively inexpensive and not require laboratory animals ***(Ausubel, et al.,1987 and Innis,et al.,1990)).*** Adisadvantage of DNA-based method is that aviable microorganism is not recovered, and therefore. further characterization is not possible.

Toxigenic and non toxigenic *P.multocida* do not differ on diagnostic biochemical reactions or morphology, Methodology for the detection of *P. multocida* toxine (PMT) includes enzyme linkd immunosorbant assay (ELISA) ***(Foged et al.,1988)***, the guinea pig skin test **(de·jong et al.,1980).**the mouse lethality test ***(Pijoan et al.,1984)*** and the cell culture in various cell lines, such as vero cells ***(Pennings & strom, 1984).*** The samples often contaminated ,and culture of *P.multocida* is occasionally unsuccessful despite use of selective agare, attempting to isolate *P.multocida* from sample can be frostruting ***(Antonio Amigot et al.,1998).*** These difficulties have led to the development of amore rapid, accurate method for detection i.e. PCR.. PCR has been particularly useful in this regard, with use of primer sequences designed to facilitate identification at any level of specificity: strain, species, genus, or all members of a domain ***(Relman and Persing , 1996).***

The gene that encodes the dermonecrotic toxin (DNT), toxA, has been isolated and sequenced ***(Buys et al., 1990; Kamps et al., 1990).*** Hybridization studies using probes of the toxA gene showed its presence in all toxigenic *P. multocida* strains and absence in most nontoxigenic strains ***(Kamps et al., 1990).*** The objective of the present investigation was (1) To determine *P. multocida* and other associated bacterial agents in pneumonic lungs of slaughtered chickens (2) To compare identification for *P. multocida* by mouse pathogenecity test and PCR technique (3)To investigate presence of toxigenic strains of *P. multocida* by PCR.

MATERIAL AND METHOD

Collection of Samples:

A total of 500 chickens showing respiratory disorder were slaughtered and examined for presence of pneumonia in lungs and 120 samples were collected from lung, heart blood and at different private farms in Egypt. The samples were obtained from five different flocks. All Samples were immediately transferred to the laboratory in sterile plastic bags.

Isolation and identification of *Pasteurella multocida according to* ***(Masdooq et al. 2008).***

Swabs from the collected samples were inoculated onto blood agar supplemented with 10% sheep blood. The plates were incubated under aerobic conditions for 24-48 h at 37c.The agar plates were checked every day and suspected colonies, were subcultured, and their Identification was attempted. The biochemical tests were performed, as described by ***(Morishita et al., 1996).***

Isolate that were Grame negative rods and catalase, oxidase,and indole positive,that fermented mannitol, that were non motile, and that did not grow in MacConkey were considered to be P.multocida.

DNA extraction:. Suspected *P. multocida* cultures were transferred into an Eppendorf tube containing 300 μL distilled water. The tubes were vortexed and incubated at 56 °C for 30 min. The suspension was then added in 300 μL of TNES buffer (20 mM Tris pH 8.0, 150 mM NaCl, 10 mM EDTA, 0.2% SDS) and 200 μg/mL Proteinase K. Following 30 min boiling, an equal volume of phenol was added to the suspension which was shaken vigorously by hand for 5 min and then, centrifuged at 11.600 g for 10 min. The upper phase was transferred into a new eppendorf tube.

Genomic DNA was precipitated with absolute ethanol and 0.3 M sodium acetate at-20 °C for one hour, or overnight. The mixture was then centrifuged at 11.600 g for 10 min and the upper phase discarded. The pellet was washed twice with 300 μL of 90% and 70% ethanol, respectively; each step was followed by 5 min centrifugation. The pellet was dried and resuspended in 50 μL sterile distilled water and used as a target DNA in PCR.

Primers. Primers used were: PMOut1 (5'AGGTGAAAGAGGTT-ATG-3') and PMOut2 (5'-TACCTAACTCAACCAAC-3') derived from Omp gene and PMTox1 (5'-GGTCAGATGATGCTAGATACTCC-3') and PMTox2 (5'-CCAAACAGGGTTATATTCTGGAC-3') (Promega) derived from *toxA* gene, respectively ***(Kamp et al., 1996; Neumann et al., 1998;).***

Polymerase chain reaction:- PCR was performed in a Touchdown Thermocycler (Hybaid, Middlesex, England) in a total reaction volume of 50 μL containing 5 μL of 10x PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 5 μL of 25 mM MgCl2, 250 μM of each deoxynucleotide triphosphate, 2U of Taq DNA Polymerase (Fermentas, Lithuania), 1 μM of each primer and 5 μL of template DNA. Amplification was obtained with an initial denaturation step at 94 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, and 56 °C for 1 min, and 72 °C for 2 min. Ten microlitres of PCR products were analysed on 1.5% agarose gel in 1XTris-Borate-EDTA (TBE) buffer. Gels were photographed under UV illumination after staining with 0.5 μg/mL ethidium bromide. Fragment sizes of 221 bp and 338 bp were verified as positive for *P. multocida* and toxigenic *P. multocida*, respectively. A 100 bp DNA ladder (Promega, Madison. WI, USA) was used as a molecular size standard.

Mouse inoculation test. according to ***(Masdooq et al.2008)*** White mice used in this study were obtained from the Animal health research institute.*P. multocida* colonies inoculated into nutrient broth.Mice divided into two group, first group (test mice) infected parenterally with 1x103 CFU per mL of *P. multocida* strain. The second group(control mice) was also inoculated with phosphate-buffered saline. Both groups of mice were observed for 48-72 h. Control mice survived, while five test mice died at the end of this time. Lung samples and Heart blood of died mice were stained with Gram and Giemsa stain and inoculated onto blood agar plates. The plates were incubated under aerobic conditions for 24-48 h at 37°Cand analysed for the presence of *P.multocida*.Suspected *P.multocida* colonies were subcultured. Isolates were identified by biochemical methods.

RESULTS

Table (1): Prevalence rate of P.multocida and other bacterial pathogen isolated from cases of Pneumonia in poultry.

Total examined poultry	Total collected samples	Positive samples	Type of infection	Micro-organism	No (%)
500	120	70	Single infection	*Pasteurella multocida*	6 (5%)
				Escherichia coli	24 (20%)
				Streptococcus spp	5 (4.2%).
				Staphylococcus spp	6 (5%)
				Pseudomonas spp	2 (1.7%).
				Proteus spp.	18 (15%).
			Mixed infection	*Escherichia coli + Staphylococcus spp*	4 (3.3%).
				Staphylococcus spp +Streptococcus spp.	2 (1.7%)
				Proteus spp. + Staphylococcus spp	3 (2.5%)+
				Escherichia coli + proteus.	1 (0.83%)

Table (2): Mouse inoculated with samples positive for *Pasteurella multocida* by culturing and PCR.

N0 of sample used	samples lethal for mice mice	samples not lethal for
6	5 (83.3%)	1(16.7)

Table (3): Comparison between PCR.and mouse pathogenicity test for randum six samples positive for *Pasteurella multocida* by culturing methods.

No of samples	Mouse pathogenicity test		PCR.		Detection of Toxigenic strains by PCR	
	+ve	-ve	+ve	-ve	+ve	-ve
5	5(80%)	0	5(100%)	0	0	5
1	0(20%)	1	1(100%)	0	0	1
Total	**5(80%)**	**1(20%)**	**6(100%)**		**0**	**6**

PCR Results. PCR amplification of genomic DNA from *P. multocida* in this study using PMOut 1-2 and PMTox 1-2 primer pairs was obtained, corresponding to the anticipated sizes of 221 bp and 338 bp, respectively.

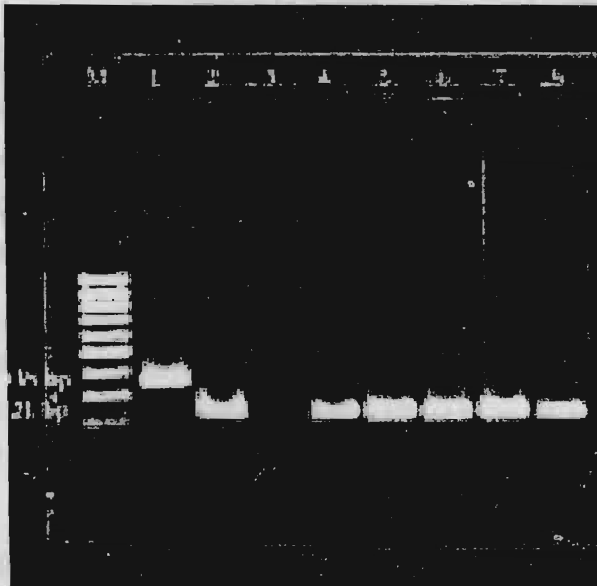

Fig. (1): PCR results of *P. multocida* isolates. (M: 100 bp DNA ladder, 1: positive control (toxigenic *P. multocida* strain), 2:positive control (*P. multocida* strain). 3: negative control, 4-9: *P. multocida* isolates.

DISCUSSION

Respiratory infections are common among the chicken population. Several micro organisms are encountered in respiratory diseases of domestic poultry ***(Ozbey, and Muz, (2006).)***. *P.multocida* is considered as enviromental pathogenic microorganisms and they are frequently encountered in both upper and lower respiratory tract specially in animal housed at bad hygienic condition ***(Quinn et al.,1994)***.

In this work bacterial growth was observed in 70 out of 120 lung samples. The isolation of *P. multocida* was in a much lower percentage 6(5%).Than that of *Escherichia coli* 24(20%).Other bacterial agent, such as *Streptococcus spp,Staphylococcus spp,Pseudomonas spp and Proteus spp.* were isolated from lung samples with percentage*5 (4.2%), 6(5%), 2(1.7%) and 18(15%)* respectively (Table 1).

The percentages *P. multocida* were rather low 6(5%) considering the total number of poultry examined. This may be due to good hygienic measure in the private farms, or the samples were not taken in the acute stage of infection, also may be due to misusing antibiotics in flocks.In addition,samples might be contaminated during the slaughtering processes.

Concerning the result of mouse inoculation test, that inoculated with strains (were both culture-and PCR-positive) (Table(2) showed that . Five (83.3%) *P. multocida* could be reported from heart blood and lung samples of dead mice. However, *P. multocida* could not be reported from one mice (16.7%).

The mouse inoculation test is often used to detect the presence of *P. multocida* in samples contaminated with other microorganisms ***(Pedrsen and Barfod.1981,and Quan et al.,1986)***.Adult male white mice injected with 1x 103CFU/ml. of *P. multocida* strain will died within 1-3 days

(Kasten, et al.,1997). However, virulence for mouse has been reported to be variable ***(Curtis et al., 1980).*** When comparing the mouse inoculation test with PCR- bassed assay (table 3), we found that of the six samples that confirmed by PCR and culture, one were not detected by mouse inoculation. It is possible that the one samples contained strains of *P. multocida* non-lethal for mice, taking into account that in the test less virulent strains are harder to detect when the mouse inoculation test is used ***(Pedrsen,K.B., and Barfod,K. 1981;Kasten et al., 1997).***

Comparing the mouse inoculation test with the PCR-based assay ***(Kasten et al., 1997)***, they found that of the 23 samples positive by mouse inoculation, 14 (61%) were not detected by the PCR-H assay.

Toxigenic and non toxigenic *P.multocida* do not differ in diagnostic biochemical reactions or morphology ***(Lichtenstelger et al., 1996).*** Therefore, additional testing of laboratory isolates is required to differentiate toxigenic and nontoxigenic strains; both in vitro and in vivo methods have been used ***(Rutter and Luther. 1984., Chanter, and Rutter. 1989., Kamps, et al., 1990., Magyar and Rimler. 1991., Foged, 1992., Nagai et al., 1994.,).*** However, culture isolation (which fails in some specimens), species identification, and toxin testing of *P. multocida* is time-consuming and costly.

A more rapid, accurate detection assay is needed for sound decisions regarding diagnosis and treatment, to prevent spread of infection into clean flocks. so the a PCR assay was adapted to differentiat toxigenic and nontoxigenic *P. multocida* ***(Nagai et al., 1994; Lichtenstelger et al., 1996; Kamp et al., 1996; Hotzel et al., 1997; Neumann et al., 1998).*** The PCR assay described by ***(Kamp et al.,1996)*** was more sensitive than the direct culture and mouse inoculation test ***(Townsend et al., 2000).***

Results of PCR (Figure 1). Illustrated that all *P. multocida* isolates that were positive by culture were also positive by PCR. In addition, no toxigenic *P. multocida* were detected in any of isolates by PCR. this result nearly agree with ***(Ozbey and Muz, 2006).***

Determination of *P. multocida* by PCR was important as some isolates were shown to be nonpathogenic for mice ***(Townsend et al., 2000).*** Now, several PCR tests have been described for *P. multocida* ***(Kasten et al., 1997; Townsend et al., 1998; Miflin and Blackall, 2001).*** Scientists reported that primers derived from Omp(Outer memberan protein) and dermonecrotic toxin (toxA) gene serve for the identification of *P. multocida* and toxigenic *P. multocida* by the PCR ***(Neumann et al., 1998; Kamp et al., 1996).*** Primer pairs PMOut and PMTox proved to be specific for *P. multocida* and toxigenic *P. multocida*, respectively. All *P. multocida* strains that were positive by culture were also positive by the PCR however toxigenic *P. multocida.* not detected from any of these samples.

Although isolation of *P.multocida* is considered to be the standard for the diagnosis of pneumonia additional testing of laboratory isolates is required to differentiate between toxigenic and non toxigenic strains.

REFERENCES

- ***Adlam C.,Rutter J.M. (1989):*** Pateurella and Pasteurellosis. Academic Press, London. 341 pp.
- ***Antonio amigot, J., Torrelfi, I. M. & Pijoan, C. (1998):*** Evaluation of techniques for the detection of toxigenic Pasteurella multocida strains from pigs J.of veterinary Diagnostic investigation 10.169-73.
- ***Ausubel,F.M.,R.Brent,R.E.Kingston,D.D.Moore,J.G.Seidman,J.A.Smith.and K.Struhl,(1987):*** Current protocols in molecular biology .John Wley & Sons, New York.

- ***Amigot,J.A.,M.Torremorell,C.Pijoan(1998):***Evaluation of techniques for the detection of toxigenic *Pasteurella multocida* strains from pigs. J. Vet. Diagn. Invest. 10, 169-173.

- ***Baldrias,L.,A.J.Frost,D.O'Boyle(1988):*** The isolation of Pasteurella-like organisms from the tonsillar region of dogs and cats. J. Small Animal Practice 29, 63-68.

- ***Buys, W. E., H. E. Smith, A. M. Kamps, E. M. Kamp, M. A. Smith (1990):*** Sequence of the dermonecrotic toxin of *Pasteurella multocida* spp. multocida. Nucleic Acids Res. 18, 2815-2816.

- ***Canal, C.W., J.A. Leao, S.L.S. Rochas, M. Macagnav,C.A.V. Lima-Rosa,S.D.Oliveira and A.Back,(2005):*** Isolation and characterization of *Ormithobacterium rhinotraechiale* from chickens in Brazil. Res. Vet.Sci., 78: 225-230.

- ***Carter, G. R. (1984):*** *Pasteurella.* Bergeys Manual of Systematic Bacteriology. Vol: I. Williams and Wilkins (Krieg, N. R. and J. G. Holt, Eds), Baltimore. pp. 552-558.

- ***Chanter, N., and J. M. Rutter. (1989):*** Pasteurellosis in pigs and the determinants of virulence of toxigenic *Pasteurella multocida*, p. 161–195. *In* C. Adlam and J. M. Rutter (ed.), Pasteurella and pasteurellosis, 1st ed. Academic Press, New York.

- ***Collins,E.M. (1973):*** Growth of *Pasteurella multocida* in vaccinated and normal mice .Infect.Immun.8:868-875.

- ***Curtis,P.E.,G.E.Ollerhead,C.E.Ellis (1980):*** Virulence and morphology of *Pasteurella multocida* of avian origin. Vet. Rec. 107, 105-108.

- ***De Jong, M. F., H. L. Oei, G. I. Tetenburg (1980):*** Atrophic rhinitis pathogenecity test for *Pasteurella multocida* isolates. Int. Pig. Vet. Soc. Proc. 6, 211. 17.

- ***Foged, N. T. (1992):*** *Pasteurella multocida* toxin. The characterisation of the toxin and its significance in the diagnosis and prevention of progressive atrophic rhinitis in pigs. Acta Pathol. Microbiol. Immunol. Scand. **100:**1–56.

- ***Foged, N. T., J. P. Nielsen, K. B. Pedersen (1988):*** Differentiation of toxigenic from nontoxigenic isolates of *Pasteurella multocida* by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 26, 1419-1420.

- ***Garlinghouse, L. E., R. F. Jr. Digiacomo, G. L. Vanhoosier, J. Jr Condon (1981):*** Selective media for *Pasteurella multocida* and *Bordetella bronchiseptica*. Lab. Anim. Sci. 31, 39-42.

- ***Hafez, M.H., (2002):*** Diagnosis of *Ornithobacterium rhinotracheale*. Int. J. Poult. Sci., 1: 114-118.

- ***Hotzel, H., W. Erler, D. Schimmel (1997):*** Detection of dermonecrotic toxin genes in *Pasteurella multocida* strains using the polymerase chain reaction (PCR). Berl Münch. Tierärztl. Wochenschr. 110, 139-142.

Innis, M. A., D. H. Gelfand, J. J. Sninsky, T. J. White (1990): PCR Protocols. A Guide to Methods and Applications. Academic Press, Inc., San Diego, Calif.

- ***Kamp, E. M., G. C. A. M. Bokken, T. M. M. Vermeulent, M. F. de Jong, H. E. C. M. Buys, F. H. Reek, M. A. Smits (1996):*** A specific and sensitive PCR assay suitable for large-scale detection of toxigenic *Pasteurella multocida* in nasal and tonsillar swabs specimens of pigs. J. Vet. Diagn. Invest. 8, 304-330.

- ***Kamps, A. M., E. M. Kamp, M. A. Smits (1990):*** Cloning and expression of the dermonecrotic toxin gene of *Pasteurella multocida* spp. multocida in Escherichia coli, FEMS Microbiol. Lett. 55, 187-190.

- ***Kasten, R. W., T. E. Carpenter, K. P. Snipes, D. C. Hirsh (1997):*** Detection of *Pasteurella multocida*-specific DNA in turkey flocks by the use of the polymerase chain reaction. Avian Dis. 41, 676-682.

- ***Knight, D. P., J. E. Paine,and D.C.Speller(1983):***Aselective medium for Pasteurella multocida and its use with animal and human specimens.J.Clin.Pathol.36:591-594.

- ***Lichtensteiger, C. A., S. M. Steenbergen, R. M. Lee, D. D. Polson, E. R. Vimr (1996):*** Direct PCR analysis for toxigenic *Pasteurella multocida*. J. Clin. Microbiol. 34, 3035-3039.

- ***Masdooq,A.A.,.Salihu,A.E Muazu,A.Habu,A.K.Ngbede,J. Haruna, G. and Sugun, M.Y.(2008).*** Pathogenic Bacteria Associated with Respiratory Disease in Poultry with Reference to Pasteurella multocida .International Journal of Poultry Science 7 (7): 674-675,

- ***Morishita,T.Y.,L.J.Lowenstine,D.C.Hirsh and D.L.Brooks, (1996):*** *Pasteurella multocida* in raptors: prevalence and characterization. Avian Dis., 40: 908-918.

- ***Magyar, T., and R. B. Rimler. (1991):*** Detection and enumeration of toxinproducing *Pasteurella multocida* with a colony-blot assay. J. Clin. Microbiol.**29:**1328–1332.

- ***Miflin, J. K., P. J. Blachall (2001):*** Development of a 23S rRNA-based PCR assay for the identification of *Pasteurella multocida*. Lett. Appl. Microbiol. 33, 216-221.

- *Moore,M.K.I.Cienjak-Chubbs,and R.J.Gates.(1994):*Anew selective enrichment procedure for isolating Pasteurella multocida from avian and environmental samples .Avian Dis.38:317-324.
- *Morris,E.J. (1958):*Anew selective enrichment procedure for isolating Pasteurella multocida from avian and environmental samples .Avian Dis.38:317-324.
- ***Nagai,S.,S.Someno,T. Yagihashi (1994):*** Differentiation of toxigenic from nontoxigenic isolates of *Pasteurella multocida* by PCR. J. Clin. Microbiol. 32, 1004-1010.
- ***Neumann, S., T. Leeb, B. Brenig (1998):*** Vergleich Zwischen PCR und Kultureller Untersuchung zum Nachweis von Infektionen mit *Pasteurella multocida* beim Hund. Kleintierpraxis 43, 69-74.
- ***Okarman,L.,I.Spanoghe,andR.M. Debruycker. (1979):*** Expermental infections of mice with Pateurella multocida strains isolated from rabbits. J.Comp.Pathol.89:51-55.
- ***Ozbey, G. and A. Muz, (2006).*** Isolation of aerobic bacteria from lungs of chickens showing respiratory disorders and confirmation of *P. multocida* by polymerase chain reaction (PCR). Vet. Archive, 76:217-225.
- ***Pennings AMMA.&strom,PK(1984):***A test in the vero cell monolayer for toxin production by strains of *Pasteurella multocida* isolated from pigs suspected of having atrophic rhinitis.Vet.Microbial9:503-508.
- ***Pedersen,K.B.,and K.Barfod.(1981).***The aetiological significance of Bordetella bronchiseptica and *Pasteurella multocida* in atrophic rhinitis of swine .Nord.Vet.Med.33;513-522.

- ***Pijoan, C., A. Lastra, C. Ramires, A. D. Leman (1984):*** Isolation of toxigenic strains of *Pasteurella multocida* from lugs of pneumonic swine. J. Am. Vet. Med. Assoc. 185, 522-523.

- ***Quan, T. J., K. R. Tsuchiya, L. G. Carter (1986):*** Recovery and identification of *Pasteurella multocida* from mammals and fleas collected during plague investigations. J. Wildl. Dis. 22. 7-12.

- ***Quinn P.J., Carter M.E., Markey B., Carter G.R. (1994):*** *Pasteurella* species. In: Clinical Veterinary Microbiology. 1st ed. Wolfe Publihings. Mosby – Year Book Europe Limited, London. 254–258.

- ***Relman, D. A., D. H. Persing (1996):*** Genotypic methods for microbial identification. In: PCR Protocols for Emerging Infectious Diseases: a Supplement to Diagnostic Molecular Microbiology: Principles and Applications (Persing, D.H.,Ed.),ASM Press. Washington.D. C. 3-31,

- ***Rutter, J.M., and P. D. Luther. (1984).*** Cell culture assay for toxigenic *Pasteurella multocida* from atrophic rhinitis of pigs. Vet. Rec. 114: 393–396.

- ***Townsend, K. M., A. J. Frost, C. W. Lee, J. M. Papadimitriou, H. J. S. Dawkins (1998):*** Development of PCR assay for species and type-specific identification of *Pasteurella multocida* isolates. J. Clin. Microbiol. 36. 1096-1100.

- ***Townsend, K. M., T. X. Hanh, D. O. Boyle, I. Wilkie, T. T. Pan, T. G. Wijewardana, N. T. Trung, A. J. Frost (2000):*** PCR detection and analysis of Pasteurella multocida from the tonsils of slaughtered pigs in Vietnam. Vet. Microbiol. 72, 69-78.

دراسات عن البكتريا المصاحبة للأمراض التنفسية في الدواجن مع الاهتمام بالباستيريلا مالتوسيدا

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معهد بحوث صحة الحيوان - المعمل الفرعى بكفر الشيخ

*قسم الدواجن المعمل الفرعي بمحافظة شمال سيناء

محـــاولات لعزل ميكروب الباستيريلا مالتوسيدا .والتأكد من حقيقة عزلة بواسطة اختبار البلمـــرة وحقن الفئران صناعيا ..لهذا الغرض تم فحص 500من الدواجن المزبوحة التى تعاني من الـــتهابات رئـــوية واخذ 120عينة رئوية مصابة .تم زراعة جميع العينات على5%Blood agar . تـــم الفحـــص البكتريولوجـــى لجميع العينات بالاضافة الى اختبار الحقن فى الفئران ووجد ان نسبة الاصـــابة بميكـــروب الباســـتيريلا مالتوســـيدا كانت 6(5%).وقد وجد ان 5(83.3%) عينات كانت ايجابيةلميكـــروب الباســـتيريلا، وسببت موت للفئران وعترة واحدة (16.3%) غير مميتة للفئران .وكانـــت جميع العينات ايجابية لميكروب الباستيريلا مالتوسيدا بواسطةالزرع وايضا بواسطة اختبار البلمرة ،فحين انة لم يتم عزل الباستيريلا المنتجة للتوكسين من اي من العينات.