RAPID IDENTIFICATION OF TOXIGENIC *PASTEURELLA MULTOCIDA* RECOVERED FROM LARGE RUMINANTS

By

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ABSTRACT

A total of 262 sample were collected as 148 nasal swabs of cows (n=82) and buffales (n=66) were collected from apparently healthy and diseased animals of different ages and 114 lung samples of cows (n=60) and buffales (n=54) were collected from apparently healthy, diseased emergency slaughtered and recently dead animals. The bacteriological investigation showed that 23 *Pasteurella multocida* suspected isolates from 262 of total samples with a percentage 8.8%. All suspected colonies (n=23) were reinvestigated using VITEK2 compact system and the results revealed isolation of 23 *Pasteurella multocida* strains. The use of molecular techniques for detection and characterization of the *Pasteurella multocida* is very important for rapid and specific detection and characterization of the organism and its virulence factor. PCR assays were used for detection of *Pastuerella multocida* toxA gene in examined samples. In the present study, *Pastuerella multocida* toxin analyzed strains showed a band at 864bp. Out of 23 positive *Pasturella multocida* isolates, only 11 *Pasteurella multocida* isolates were proved to be toxigenic strains with incidence of 47.8%. In conclusion, toxigenic strains of *Pasteruella multocida* play an important role in its pathogenesis.So differentiation of toxigenic and non-toxigenic strains of *Pasteurella multocida* is essential for the accurate diagnosis, treatment, and prevention of diseases caused by *Pasteurella multocida.* Using PCR is highly sensitive and specific for toxA gene detection.

Keywords:

Pasteurella multocida, large ruminants, Vitek2 system, tox A gene, PCR.

INTRODUCTION

Pasteurella multocida is a pervasive bacterium that can be part of the normal upper respiratory tract flora of many animal species **(Dabo *et al.,* 2007).** The bacterium can cause mild chronic upper respiratory tract inflammation, acute pneumonia or septicemia **(Wilson and Ho, 2013).**

Pasteurella multocida is a Gram-negative bacterium responsible for Pasteurellosis (acute septicemic disease characterized by high morbidity and mortality rate as well as severe economic losses) in cattle, buffalo, sheep, goat and poultry **(Jakeen *et al.,* 2016).** All age groups are affected with *Pasteurella multocida*, but calves of 6 months to 2 years more susceptible and representing 14% of respiratory diseases in calves **(Radostits *et al.,* 2007).** Several host and pathogen-specific attributes do determine the outcome of infection caused by *Pasteurella multocida* **(Verma *et al.,* 2013).** By itself, this bacterium dose not usually cause serious disease, but it can be a significant pathogen if associated with other bacteria, viruses, or Mycoplasma as predisposing factor when calves are stressed **(Ishiguro *et al.,* 2005)**. The outbreak of *P. multocida* showed acute respiratory disease manifested with high fever, nasal discharge, respiratory distress, polypnoea and death within few days. The postmortem findings showed severe congestion in the lung, trachea, liver and small intestine **(Azizi *et al.,* 2011)**. The diagnosis of Pasteurellosis in farm animals is still under taken by the clinical manifestation, post mortem finding and by conventional bacteriological methods for identification of the causative agents, such methods are not reliable and time consuming and at the same time it is very difficult to differentiate between *P. multocid*a and the other *Pasteurella* species specially the *Pasteurella haemolytica* (*Mannheimia haemolytica*) **(Berge *et al.,* 2006; Bell, 2008).** The technique of VITEK2 has improved the field of bacterial screening by providing a much faster, more reliable cheaper and highly sensitive technique for bacterial identification **(Wallet *et al.,* 2005).**Toxigenic *Pasteurella multocida* is unintentionally spread to uninfected herds via the addition of asymptomatic, and many aspects of toxigenic *Pasteurella multocida* epidemiology and ecology remain unknown, in part, because of the lack of a rapid, sensitive assay to confirm infection. False negative culture results occur when *Pasteurella multocida* dies during transportation to the laboratory or is overgrown by other bacteria (nasal flora and contaminants) in the culture **(De Jong, 1992).** Toxigenic and non-toxigenic *Pasteurella multocida* isolates do not differ on diagnostic biochemical reactions or morphology. Additional testing of laboratory isolates is required to differentiate toxigenic and nontoxigenic strains: both in vitro and in vivo methods have been used **(Nagai *et al.,* 1994).** However, culture isolation (which fails in some specimens), species identification, and toxin testing of *Pasteurella multocida* are time-consuming and costly. A more rapid, accurate detection assay is needed for rapid diagnosis and treatment, to prevent unintentional introduction of infected animals into clean herds, to support basic studies in ecology and

epidemiology of the organism. Assay based on PCR are contributing to diagnostic microbiology **(Altwegg and Verhoef, 1995)**. The conversation of the sequence of the toxA gene indicates that assays for the gene, including PCR, are valid for the identification of toxigenic isolates of *Pasteurella multocida* **(Buys *et al.,* 1990 and Peterson, 1990).** This study was aimed to isolate and identify *Pasteurella multocida* strains using rapid and accurate technique (Vitek2 compact system) and differentiate between toxigenic and non-toxigenic isolates using polymerase chain reaction (PCR).

MATERIAL AND METHODS

Samples:

A nasal swab is used to sample both sides of the nasal cavity and should then be placed in a non-nutritive transport medium (e.g. phosphate buffered saline) and kept at 4-8°C during transition to avoid overgrowth by other faster-growing bacteria. Transition time not exceed 24 hours.Pneumonic lungs were collected from freshly dead cattle and buffalo which had a history of severe respiratory distress, pyrexia, and nasal mucopurulent discharge.Samples were packed separately in sterile plastic bags, labeled and transferred to the laboratory in ice box.

1-Bacteriological examination:

Nasopharyngeal swabs were inoculated into casein sucrose yeast (CSY) broth for 6-8 hours, and then a loopful was cultivated onto casein sucrose yeast (CSY) agar, sheep blood agar and MacConkey agar.

-Organs specimens were cultured directly onto the previously mentioned media. All agar plates were incubated at 37°C for a minimum of 48 hours. Suspected colonies were picked up for morphological and biochemical identification **(Quinn *et al.,* 2002)** as traditional method of identification and reinvestigated by Vitek2 compact.

2-Identification of *Pasteurella multocida* using VITEK2 compact system (according to the manufacture structure biome'rieux 2006 and Sahar *et al*., 2014):

-**Suspension preparation:** A sterile swab was used to transfer a sufficient number of suspected colonies of pure culture to suspend in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, PH 4.5 to 7.0) in a 12x75 mm clear plastic (polystyrene) test tube and then mix well. The turbidity was adjusted to the equivalent of 0.5-0.63 McFarland turbidity with a VITEK2 instrument DensiChee **(biomerieux)**.

Inoculation: for each isolate, the identification Gram negative (GN) cards were inoculated with microorganism suspension. The card was identified by different 47 biochemical tests. A test tube containing the microorganism suspension was placed into a special rack (cassette). The filled cassette was placed into a vacuum chamber station. After the vacuum is applied and air is reintroduced into the station, the organism suspension was forced through the transfer tube into microchannels that fill all the test wells.

Card sealing and incubation: inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. All cards were incubated on line at 35.5^{o}C ± 1.0 C for approximately 6 hours. During incubation the cards were read every 15 min automatically. The final results were obtained automatically printed within 6-8 hours. All used cards were automatically dispensed into waste container.

3-Detection of the toxA Gene by PCR (Tang *et al*. 2009):

The PCR assay was carried out on the tested toxigenic strains of *Pasteurella multocida* as follow.

<u>DNA extraction:</u>

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the isolate was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56C for 10 min. After incubation, 200 µl of 100 % ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

<u>Oligonucleotide Primer:</u>

Primers used were supplied from **Metabion (Germany)** are listed in table below.

<u>PCR amplification:</u>

Primers of tox Aware utilized in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied bio system 2720 thermal cycler.

<u>Analysis of the PCR Products:</u>

The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100 bp plus DNA Ladders

(Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Anne a-ling	Extension		
toxA	CTTAGATGAG CGACAAGG GAATGCCACA CCTCTATAG	864	94˚C 10 min.	94˚C 1 min.	48˚C 1 min.	72˚C 1 min.	72˚C 10 min.	Tang *et al.* 2009

RESULTS

Bacteriological investigation of 262 different samples revealed 23 suspected colonies of *pasteurella multocida* .Identification using Vitek 2 compact showed isolation of 23 *Pasteurella multocida* isolates with an incidence of 8.8% as Showed in (Table 1)., detection of tox A gene by using polymerase chain reaction (PCR) revealed that tox A gene found only in 11 isolate with an incidence of 47.8% as showed in Fig. (1)

Table (1): positive sample of *Pasteurella multocida* isolated from nasal swab and lung tissue of cattle and buffalo.

Total Sample	No. of nasal swabs (148)		No. of lung tissue (114)		Total
Animal	Cattle	Buffalo	Cattle	Buffalo	
Examined samples	82	66	60	54	262
+ve sample	5	3	8	7	23
%	6.0%	4.5%	13.3%	13.0%	8.8%

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATK	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Sheet (1).Biochemical identification of *Pasteurella multocida* by Vitek2 compact

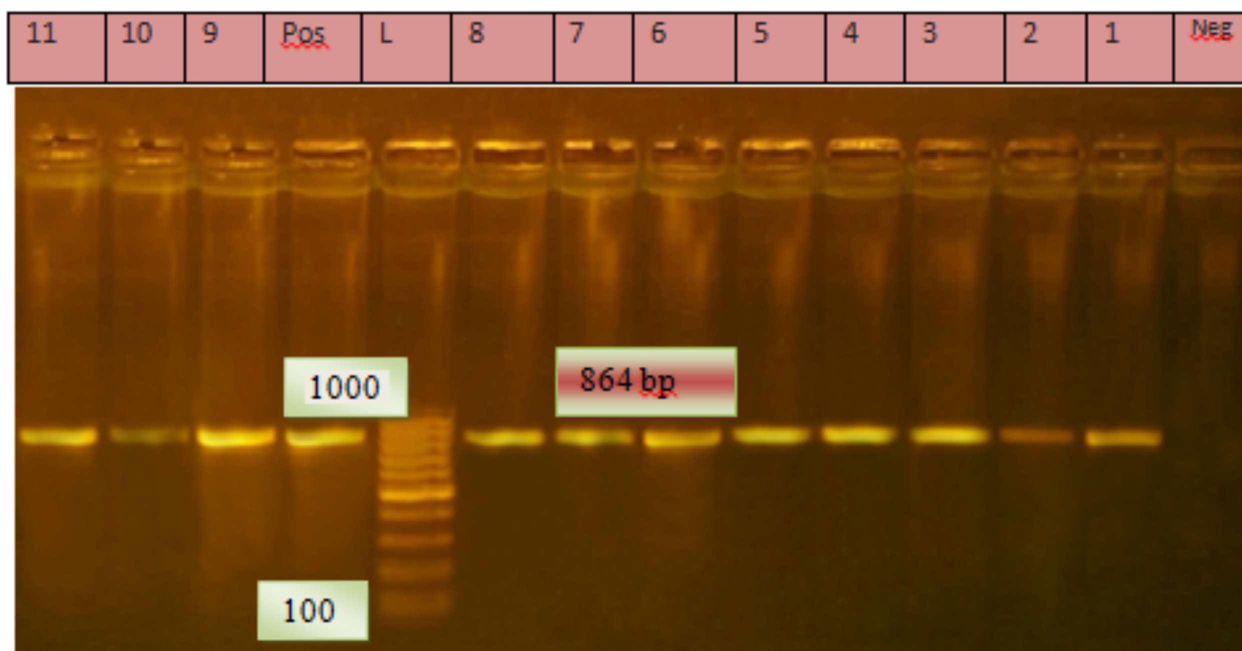

Fig. (1): Agarose gel electrophoresis of PCR for confirmation of tox A gene of *Pasteurella multocida* isolates. **Lane L:** 100-1000 bp ladder as molecular size (DNA marker). **Lane Pos.:** Control positive *Pasteurella multocida*. **Lane Neg:** Control negative. **Lanes from 1 to 11:** Positive *Pasteurella multocida* toxA gene (864 bp).

DISCUSSION

Pasteurella multocida is an important pathogen hazardous to animal and human beings with worldwide distribution causing high economic losses **(Hung *et al,* 2010).** *Pasteurella* is a type of bacterial that commonly infects the respiratory tract of ruminants causing respiratory disease and it is one of the most common bacteria isolated from animals suffering from pneumonia. *Pasteurella* is usually a secondary bacterial invader, meaning that a virus or some other disease first weakens the immune system thus allowing *Pasteurella* to invade. *Pasteurella multocida* is considered a part of the normal bacterial flora of the upper respiratory tract of most ruminants and can cause disease when it is inhaled into the deeper portions of the respiratory tract and the animal's normal defense system is impaired. Transmission of these bacteria is especially efficient when animals are crowded (as in shipment) or closely confined **(Smith,2009).** Preliminary identification of *Pasteurella multocida* isolates was carried out according to standard biochemical tests as described by **(Quinn *et al*., 2002)**. The isolates were gram negative coccobacilli produce indole, catalase and oxidase but fail to use citrate, Methyl red (MR), Voges-Proskauer (VP) and gelatin liquefaction. They do not produce hydrogen sulphide and urease. They do not grow on MacConkey agar; do not show hemolysis on blood agar. The CSY agar containing 5% blood agar was suitable for its growth. In this study, prevalence of *Pastuerella multocida* in examined small ruminants (apparently healthy, pneumonic and dead) was detected. Suspected colonies showed typical morphological characters of *Pasteurella multocida* on CSY agar containing 5% blood medium (a suitable medium for the growth of *Pasteurella multocida* and no growth on MacConkey agar, submitted to for further microscopically examination. Isolates showed Gram negative coccobacilli, occur singly, in pairs or less frequently in short chain were identified using Vitek2 compact system (Sheet, 1). Bacteriological investigation of 262 different samples revealed 23 suspected colonies of *pasteurella multocida* strains with incidence 8.8%, The incidence were recovered by **Sedeek and Thabet (2001)**, **Defra (2006) and Sayed and Zaytoun (2009)** who isolated *Pasteurella multocida* in an incidence of 8.3 %, 15% and 15.89 % respectively the higher incidence obtained by **Enany *et al*., (2012) and Khadr (2005)** who isolated *Pasteurella multocida* with incidence of 19.23 % and 22.73% respectively. All suspected colonies (23) were reinvestigated using VITEK2 compact system and the results confirm isolation of 23 *Pasteurella multocida* isolates with an incidence of 100 %. A confirmatory identification by Vitek2 compact system using Gram negative card is based on established biochemical

methods and newly developed substrates measuring carbon source utilization, enzymatic activities and resistance was employed. The identification card contains 47 different biochemical tests. Final results are available in approximately 6-8 hours **(Biome'rieux user guide, 2006).** VITEK2 system is a promising, highly automated new tool for the rapid identification of gram-negative bacilli isolated from clinical specimens **(Farid *et al*., 2013).** Other advantages of the VITEK 2 system are the decreased turnaround and hands-on times since the system is nearly fully automated. **(Mary, *et al.,* 2009).** Differentiation of toxigenic and non-toxigenic strains of *Pasteurella multocida* is essential for the accurate diagnosis, treatment, and prevention of diseases caused by *pasteurella multocida*. Several tests are used to detect toxigenic strains, including mouse lethality and in vitro cytotoxicity assays and ELISA **(Foged *et al.,* 1988)**. A differential test that does not require live animals or the time and labor involved with cell culture is needed so PCR assay was applied for the detection of *Pastuerella multocida* toxA gene in tested isolates, one represent the isolates recovered from pneumonic cattle and buffalo and the other was isolated from apparently healthy animals. PCR could avoid potential problems such as the suppression of the expression of a gene. Moreover, **Nakai *et al.,* (1984)** suggest PCR as a fast and safe alternative for toxin detection in *Pasteurella multo-cida* strains. Moreover**, Nagai *et al.,* (1994)** stated that, the advantages of PCR compared with existing tests include: high sensitivity and specificity, simplicity of the procedure without the need for cell culture laboratory animals, rapidity of the test and safety as the result of the avoidance of handling live bacteria (only DNA is handled). *Pastuerella multocida* toxin analysed strains showed a band at 864bp Fig. (1), these results agree with **Lichtensteiger *et al.,* (1996).** Many authors, **Ewers *et al.,* (2006), Shayegh *et al.,* (2010) and Khamesipour *et al.,* (2014)** recorded toxA genes in *Pastuerella multocida* isolates. In conclusion, VITEK2 system is a promising, highly automated new tool for the rapid identification of gram-negative bacilli isolated from clinical specimens.Toxigenic strains of *Pasteruella multocida* play an important role in its pathogenesis,so differentiation of toxigenic and non-toxigenic strains of *Pasteurella multocida* is essential for the accurate diagnosis, treatment, and prevention of diseases caused by *pasteurella multocida*.Using PCR was highly sensitive and specific for toxA gene detection.PCR compared with existing tests include: high sensitivity and specificity, simplicity of the procedure without the need for cell culture laboratory animals, rapidity of the test and safety as the result of the avoidance of handling live bacteria (only DNA is handled).

REFERENCES

Altwegg, R.M. and Verhoef, J. (1995): Amplification methods in diagnostic microbiology. Elsevier Science, Amsterdam.

Azizi, S., Tajbakhsh, E., Rezaii, A., Nekouei, S., Namjoo, A.R. (2011): The role of Mycoplasma ovipneumoniae and Mycoplasma arginini in pneumonic lungs of slaughtered sheep. Rev. Med. Vet. 162 (6), 310 - 315.

Berge, A.C.B., Sischo, W.M., Craigmill, and A.L. (2006): Antimicrobial susceptibility patterns of respiratory tract pathogens from sheep and goats. J. Am. Vet. Med. Assoc. 229 (8), 1279-1281.

Bio Me´rieux (2006): Vitek2 product information, document 510769-4EN1. biome'rieux, Inc., Durham, NC.

Buys, W.E.C.M.; Smith, H.E.; Kamps, A.M.I.F.; Kamp, E.M. and Smits, M.A. (1990): Sequance of the dermonecrotic toxin of *Pasteurella multocida* ssp. *multocida.* Nu-cleic Acids Res. 18:2815-2816.

Dabo, S.M.; Taylor, J.D. and Confer, A.W. (2007): *Pasteurella multocida* and bovine respiratory disease. Anim. Health Res. Rev. 8 (2): 129 -150.

De Alwis, M.C.L. (1992): Haemorrhagic septicaemia-a general review. Br. Vet. J., 148, 99-112.

Defra (2006): Veterinary Investigation Surveillance Report (VIDA). www.defra.gov.uk/vla/reports/docs/rep_vida_cattle99_06 .

Enany, M.E.; Riad, E.M. and Wahdan, A. (2012): A Bacterial causes of pneumonia in buffalo calves SCVMJ, XVII (2): 27-38.

Ewers, C.; Lubke-Becker, A.S. Bethe, A. Kiebling, S. Filter, M. and Wieler, L.H. (2006): Virulence genotype of *Pasteurella multocida* strains isolated from different hosts with various disease status. Veterinary Microbiology vol. 114, 1ssues 3 - 4 May, P. 304 -314.

Farid, A.F.; Saher, R. Mohamed; Aziz, H.M. and Amany, N. Dapg (2014): Rapid identification of Gram negative bacteria isolated from mastitic cow's milk in different dairy farms by Vitek2 compact system. Animal Health Research Journal Vol. 2 No.1 Special Issue for second Scientific Conference 2-6 February.

Fillion, L.G.; Cho, H.J.; Shewen, P.E.; Raybould, T.J.G. and Wilkie, B.N. (1985): Comparison of seriological techniques to measure antibody to *Pasteurella haemolytica* A 1. Can. J. Comp. Med. 49: 99-103.

Hung, Y.; Hung, X.; Tian, M.; Zoa, N.; Coa, S. and Wen, X. (2010): Identification and sequencing of two expression genes of por-cine *Pasteurella multocida* in vivo. J. of Ani-mal and Veterinary advances 9 (3): 441 - 445.

Ishiguro, K.; Kitajima, T.; Kubota, S.; Amimoto, K.; Oda, K.; Fukuyama, S. and Shimizu, Y. (2005): Experimental infection of calves with *Pasteurella multocida* serovar A: 3 isolated in Japan. J Vet Med Sci, 67, 817-819.

Jakeen, K.E., Samah, S.A., Soumaya, A.E., Ashgan, M.H., Abdullah, A.A., Moussa, I.M., 2016. Comparative studies for serodiagnosis of haemorrhagic septicaemia in cattle sera. Saudi J. Biol. Sci. 23, 48-53.

Khadr, A.M. (2005): Outbreaks of pneumonia in beef calves associated with bovine viral diarrhea virus seroconversion and other respiratory pathogens. Beni-Suef Vet Med J., 15 (2): 289-294.

Khamesipour, F.; Momtaz, H. and Ma-moreh, M.A. (2014): Occurrence of virulence factors and antimicrobial resistance in *Pasteurella multocida* strains isolated from slaughter cattle in Iran. Frontiers in Microbiology. October, vol. 5 Article 536 P. 1-9.

Lichtensteiger, C.A.; Steenbergen, S.M.; Lee, R.M.; Polson, D.D. and Vimr, E.R. (1996): Direct PCR analysis for toxigenic *Pasteurella multocida.* J. Clin. Microbiol. 34 (12): 3035.

Mary, F.;Alauzet, C.and Rieqely,P.(2009): Evaluation of the new vitek2 ANC card for identification of medically relevant anaerobic bacteria. J. Clinical .Microb. June 47 (6): 1923-1926.

Nagai, S.H.; Someno, S. and Yagihashi, T. (1994): Differentiation of toxigenic from non-toxigenic isolates of *Pasteurella multocida* by PCR. Journal of Clinical Microbiology Vol. 32, No. 4 P. 1004-1010.

Nkai, T.; Sawata,A.;Tsuji, M.; Samejima, Y. And Kume, K. (1984): Purification of Dermonecrotic Toxin from a Sonic Extract of *Pasteurella multocida* SP-72. Serotype D. Infection and immnunity, p. 429 - 434

Peterson, S.K. (1990): The complete nucleotide sequence of the *Pasteurella multocida* toxin gene and evidence for a transcriptional repressor, Txar. Mol. Microbiol. 4: 821-830.

Quinn, P.J.;Carter,M.E.;Markey, B.K. and Carter, G.R. (1994): Bacterial pathogens: Microscopy, culture, and Identification. In Clin. Vet. Microbiol. London: Wolfe Publishing; 1994: 21-60.

Radostits, O.M.; Gay, C.C.; Hinchcliff, K. W. and Constable, P.D. (2007): Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 10. ed. Philadelphia: Saunders, 2007. P.724-725.

Sahar, R. Mohamed; Ibrahim, E.M.; M.Makin El-Bardisy; Aziz, H.M.; Amany, N.Dapgh and Hala F. Habashy (2014): Evaluation of the automated VITEK2 compact system in the identification of Gram positive Cocci in comparison to the conventional identification methods .Animal Health Research Journal Vol., 2 (4): 37- 48.

Sayed, S.M. and Zaitoun, A.M.A. (2009): Aerobic bacterial pathogens of pneumonic feedlot buffalo-calves, IN Assuit Governorate, Egypt Ass. Univ. Bull. Environ. Res. Vol., 12 (1):55-62.

Sedeek, S.R. and Thabet, A.M. (2001): Some studies on bacterial causes of pneumonia in cattle in Assuit Governorate. Assuit Vet. Med. J. 45 (90): 243-255.

Shayegh, J.; Atashpaz, S. and Salehi, T.Z. (2010): Potential of *Pasteurella multocida* isolated from healthy and diseased cattle and buffaloes in induction of diseases. Bull Vet Inst Pulawy 54, 299-304

Smith, B.P. (2009): Large Animal Internal Medicine, 3rd Ed. Mosby-Elsevier Publishing. St. Louis, MO. 2009. pp. 559-561.

Ta Ng, X., Z. Zhao, J. Hu, B. Wu, X. CAI, Q. He, H. Chen (2009): Isolation, antimicrobial resistance and virulence genes of *Pasteurella multocida* strains from swine in China. J. Clinic. Microbiol. 47, 951-958.

Verma, S.; Sharma, M.; Katoch, S.; Verma, L.; Kumar, S.; Dogra, V.; Chahota, R.; Dhar, P. and Singh, G. (2013): Profiling of virulence associated genes of *Pasteurella multocida* isolated from cattle. Veterinary Research Communications March 2013, Vol-ume 37 (1): 83 - 89.

Wallet, F.; Loiez, C; Renaux, E. and Courcol, R. (2005): Performances of VITEK 2 colori-metric cards for identification of gram positive and gram negative bacteria. J. Clinical Microbial .43. 4402-4406.

Wilson, B. A. and Ho, M. (2013): *Pasteurella multocida*: from zoonosis to cellular microbioly. Clin Microbiol Rev 26, 631-655.