Bacterial Agent of Respiratory Manifestation in Cattle and The Associated Biochemical Alterations in Menoufiea Governorate

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ABSTRACT This study was carried out on a feedlot cattle farm at Menoufiea governorate suffering from respiratory distress, fever, with mild to continuous coughing, loss of appetite, nasal discharge, depression and reduction in fertility. The morbidity rate was 25 % and the mortality rate reached 4%. Mycoplasma was suspected to be incriminated as causative agent. Nasopharyngeal swabs and blood samples were taken from the diseased animals. Bacteriological examination of recovered isolates revealed that *Mycoplasma bovis* (20%) and this was confirmed by Polymerase Chain Reaction (PCR).The studied biochemical parameters revealed a significant decreased in serum total protein, albumin, total globulin and consequently in A/G ratio. Liver enzyme alanine aminotrasferase (ALT) was significantly decreased while aspartate aminotransferase (AST) did not change. [Nature and Science 2009;7(9):26-30]. (ISSN: 1545-0740).

KEY WORDS: Mycoplasma, PCR, Serum protein, AST, ALT

1-INTRODUCTION

Bovine respiratory disease (BRD) complex is the most important cause of mortality and culling of weaned calves and young animals after arrival at a feedlot (Kelly and Janzen ,1986). Infectious agents implicated in bovine respiratory disease include viruses, bacteria, mycoplasma and chlamydia. Many viruses have been associated with respiratory disease in cattle, many acting in conjunction with bacteria to produce severe pneumonia (Radostits, et al., 1994). Mycoplasmas are prokaryotes which lack a true cell wall and are known to cause chronic diseases in man and animals. Mycoplasma bovis is associated with a variety of bovine diseases, including pneumonia, polyarthritis, tenosynovitis and mastitis, which cause considerable economic losses (Henderson and Ball, 1999).

The infection is usually introduced to M. bovis-free herds by clinically healthy calves or young cattle shedding the mycoplasma and once established on multi-age sites it becomes very difficult to eradicate. Its appearance on some farms suffering low grade respiratory disease can lead to increased morbidity and mortality (Gourlay et al., 1989). Infected cattle shed the mycoplasma via the respiratory tract for many months and even years where they act as reservoirs of infection (Pfutzner, 1990). Contact animals become infected via the respiratory tract, the teat canal or genital tract; artificial insemination with infected semen is another common route (Pfutzner, 1990). The male genital tract can become infected with M. bovis through contact with other animals or, possibly, via a heavily contaminated environment.

This study aimed to diagnose the cause of respiratory affection in a farm of feedlot cattle, together with monitoring the biochemical changes associated with the causative agent.

MATERIAL AND METHODS

A feedlot farm at Menofia governorate suffering from respiratory distress, mild to continuous coughing, loss of appetite, nasal discharge and depression with morbidity rate 25 % and mortality rate 4%. Vaccination program against viral diseases was maintained together with strict internal and external parasite control. Nasal swabs were taken from 15 of both diseased and apparently healthy animals for identification of the causative microorganism, also blood samples were taken for biochemical investigation.

1-Isolation of Mycoplasma: The samples were cultured on Modified HayFlicks medium (Rosendal, 1994) and B.H.S.L medium (Carmicheal et al., 1972) as described by (Sabry and Ahmed 1975). Digitonin sensitivity test as described by (Freundt et al., 1973) was done to differentiate between genus Mycoplasma and Acholeplasma, where genus Mycoplasma was digitonin sensitive and Acholeplasma was digitonin resistant, (Thurmond et al., 1989). Biochemical characterization was carried to differentiate the purified Mycoplasma isolates using different biochemical tests as glucose fermentation test, arginine test (Sabry, 1968) , and

film and spots formation (Cottew, 1983). Serotyping was carried by growth Inhibition test (GI), according to (Clyde 1964) with reference antisera.

2-Polymerase chain reaction (PCR):

a) Preparation of samples for DNA extraction (Yleana et al., 1995): 5ml of a 24 hour broth cultures of isolates were centrifuged for 10 minutes at 12000 r.p.m. The pellet was washed twice in 1 ml of PBS pH 7.2 and suspended in 50 µl PBS. The cell suspension was heated directly at 100oC for 10 min. in a heat block to break the cell membranes, and then cooled on ice for 5 min. Finally, the cell suspension was centrifuged for 5 min. and the supernatant containing chromosomal DNA was collected and stored at -20^{o}C until used.

b) Primer selection (Yleana et al., 1995): Two oligonucleotide primers were selected for the detection of M. bovis. The sequence of the primers was (prepared by Sigma):

Forward:5-CCTTTTAGATTGGGATAGCGGATG-3

Reverse:5-CCGTCAAGGTAGCATCATTTCCTAT-3

Procedure for DNA amplification: PCR amplification was performed in 50 µl reaction mixture consisting of 5 µl of 50 ng M. agalactiae 90 min. at 100 volts, DNA Ladders: 100 bp (Pharmacia), Cat. No. 27-4001- 01, USA was added then stained with ethidium bromide. After electrophoresis, the gel was visualized by UV transillumination and photographed. Arthritis and reduced weight gain in calves, mastitis in cows and reproductive problems in both cows and bulls (Romváry et al., 1977; Kreusel et al., 1989; ter Laak et al., 1992; Pfützner and Sachse, 1996; Nicholas and Ayling, 2003). It can occasionally be involved in other diseases such as meningitis (Stipkovits et al., 1993), otitis media (Walz et al., 1997) and abortion (Byrne et al., 1999).

This study was made on a feedlot farm at Menofia governorate suffering from respiratory distress with mild to continuous coughing, loss of appetite, nasal discharge and depression. The morbidity rate was 25 % and the mortality rate reached 4%. The nasal swabs (n=20) taken were examined for bovine respiratory viruses, bacteria but DNA, 10 µl of 10 x Taq buffer (10mM tris- HCl [pH 8.8], 50 mM KCl), 1 µl of 50 pM of each primer, 1.5 mM MgCl2, 1 µl of 2U of Taq thermostable DNA polymerase, 1 µl of 50 uM of each dNTP, and 31µl of DNase- RNase- free, deionized water. The thermal profiles were as follows: Denaturation at 94 oC for 45 seconds, primer annealing at 60oC for 1 min., and extension at 72oC for 2 min.the amplifications were performed for 30 or 35 cycles with a final extention step at 72oC for 3 min. After the reaction, the amplified DNA was electrophoresed on 1.5% agarose gel for

3-Biochemical tests**:** serum total protein concentration was detrmined according to Sannenwirth and Jarett (1980), albumin concentration was made according to the methods described by Drupt (1974) and serum globulin detected by subtraction. Serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were made as recorded by Reitman and Frankel (1957).

Results and Discussion

M. bovis is an important but sometimes overlooked pathogen in cattle. It causes major economic losses mainly by causing pneumonia,

Table (1) showed the recovery rate of mycoplasma from nasal swabs as 20% (4 out of 20) and Acholeplasma spp was found to be 5% one out of 20. In other studies the recovery rate was13% (El-Shater and Eissa, 2001); 63 out of 432 calves (15%) via culture (Wiggins et al., 2007) and 31.58% (Eissa et al., 2007).

The obtained isolates were subjected to the polymerase chain reaction (PCR) for confirmation of the results using the 16S rRNA gene for M. bovis. It was found that the obtained 4 Mycoplasma isolates were positive to the used gene giving positive band at 360 bp, so the isolates confirmed as *M. bovis* (image 1). A similar results were obtained by Hotzel et al. (1996); Ghadersohi et al. (1997); El-shater and Eissa (2001); Susan (2006) and Eissa et al. (2007).

Table (1) recovery rate of mycoplasma isolated

Sample	Primary isolation				Expected type
	No Examined	No. +ve recovered	Digitonin	%+ve	
Nasal swabs	20	4	+ve	20%	Mycoplasma Spp
		1	-ve	5%	Acholeplasma spp

M. bovis infection represents a major disease burden for cattle producers worldwide, emphasizing the need for a reliable method of molecular typing for outbreak investigation and epidemiological surveillance (McAuliffe et al, 2004). Chavez et al., (1995) reported that the use of PCR makes the identification of *M. bovis* much shorter comparing to the conventional culture methods.

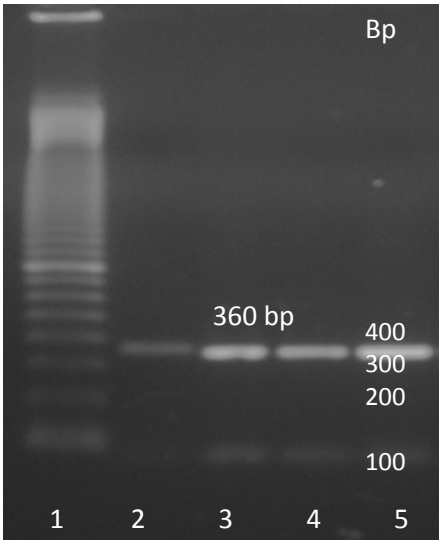

Image (1) the agarose gel electrophoresis of the obtained isolates of *M. bovis*

1- 100bp marker
2- 5- *Mycoplasma bovis* isolates

The taken serum samples were used for the studying the biochemical changes accompanying the case. In table (2), it was found that mycoplasma infection cause a significant decrease in total protein, albumin, total globulin and consequently in A/G ratio. Also the liver enzyme alanine aminotransferase (ALT) was significantly decreased while aspartate aminotransferase (AST) did not changed.

The decrease noted in total protein caused by the decrease both in albumin and globulin, a similar finding were recorded by Eissa et al. (2007). Science hypoalbuminemia may be due to increased catabolism as a result of tissue damage or inflammation as stated by Limidi and Hyde (2003), that agreed with Wise and Evans (1975), who mentioned that serum albumin concentrations, were markedly reduced in poults with *M meleagridis* and also, suggested that low serum albumin concentrations may play a primary role in the pathogenesis of mycoplasma. UIvund (1990) observed hypoalbuminaemia in mycoplasma mastitic cows and revealed it to the inadequate protein synthesis as a result of Mastitis which badly affected the hepatic parenchyma leading to the failure of protein synthesis as recoded by Coles (1986).

Immunosuppression caused by *M. bovis* had been observed by the results obtained by Thomas et al. (1986) who recorded low levels of IgA antibody in sera three and four weeks after Mycoplasma infection, and Postepski et al. (2003) who found low titer of IgG and IgM with patients of *Mycoplasma pneumonia.*

Table (2): The biochemical changes accompanying *Mycoplasma bovis* infection

Items tested	control	diseased	LSD
Albumin (mg %)	3.74±0.11 a	2.60±0.14 b	0.4419***
Total globulin (mg %)	4.10±0.11a	3.160±0.260b	0.688*
Total protein (mg %)	7.83±0.20 a	5.74±0.268b	0.8179***
AG Ratio	0.91±0.02 a	0.737±0.096b	0.141*
ALT (u/L)	58.25±6.76 a	30.00±4.34 b	19.661*
AST (u/L)	142.75±15.69	121.00±14.40	52.121ns

*: Significant variation between groups by one ways ANOVA at $P \leq 0.05$.

Serum ALT decrease may be due to alternation in the metabolic rate resulted from nutritional deficiency in mastitic buffaloes as reported by UIvund (1990) and Abd El- Ghany et al. (2007). On the other hand mycoplasma inside cells produces ammonia and oxidized compound (hydrogen peroxide, peroxide compound) which all toxic to cells (Nicholson et al., 1998).Rao and Murthy 1992 stated that activities of ALT decreased in hyperammonemic states.

Mycoplasma infection in cows may cause serious problems in herd not only due to respiratory distress but also to the immunosuppressive effect that may lead to combined infection.

References

1 Abd El- Ghany, S; Mohsen, D. and El-Shafey, D. (2007): A comparative Study on Diagnosis of mycoplasmal mastitis As And Interrelated to clinicopathological changes. Vet.Med.J.,55 (4): 1019-1033.

2 Byrne, W. J.; Brennan, P.; McCormack, R. and Ball, H. J. (1999): Isolation of *Mycoplasma bovis* from the abomasal contents of an aborted bovine fetus. Vet Rec.; 144: 211-212.

3 Chavez Gonzalez, Y. R.; Ros, B. C. ; Bolske, G.; Mattsson, J. G.; Fernandez, M. C. and Johansson, K. E. (1995): In vitro amplification of the 16S rRNA genes from *Mycoplasma bovis* and Mycoplasma agalactiae by PCR. Vet. Microbiol. 47, 183-190.

4 Drupt, F. (1974): Calorimetric determination of serum albumin using bromocresol green at pH 4.2. Pharma. Boil., (9) 77-82.

5 Eissa, S. I., Moussa, S.Z., Hanaa A. A., and El-Maedawy, S. A. (2007): Oxidative Stress and Immunosuppression Induced by Mycoplasma Infection in Cattle. The Egypt. J. Bioch. Mol. Biology, (25): 62-77.

6 El-Shater, S. A. and Eissa, S. I. (2001): The use of polymerase chain reaction (PCR) technique for the rapid detection and identification of Mycoplasma bovis. Vet. Med. Ass.: 61 (2):427-432.

7 Ghadersohi, A.; Coelen, R. J.; and Hirst, R. G. (1997): Development of a specific DNA probe and PCR for the detection of Mycoplasma bovis. Vet. Microbiol., 56:87-98.

8 Gourlay, R.N.; Thomas, L.H. and Wyld, S.G. (1989): Increased severity of calf pneumonia associated with the appearance of *Mycoplasma bovis* in a rearing herd. Veterinary Record, 124:420–422.

9 Henderson, J. P. and Ball, H. J. (1999): Polyarthritis due to *Mycoplasma bovis* infection in adult dairy cattle in Northern Ireland. Vet. Rec. 145, 374-376.

10 Hotzel, H.; Sachse, K. and Pfützner, H. (1996): Rapid detection of *Mycoplasma bovis* in milk samples and nasal swabs using the polymerase chain reaction. J. Appl. Bacteriol., 80: 505-510.

11 Kelly, A.and Janzen, E. (1986): A review of morbidity and mortality rates and disease occurrence in North American feedlot cattle. Can. Vet. J.;27:496-500.

12 Kreusel, S.; Bocklisch, H.; Pfützner, H.; Brys, A.; Leirer, R. and Ziegenhals, U. (1989): [Experimental infections of bulls with Mycoplasma (M.) bovis and M. bovigenitalium]. Arch. Exp. Veterinärmed. 43, 705-712.

13 Limdi, J. K. and Hyde, G. M. (2003): Evaluation of abnormal liver function tests. Postgrad. Med. J., 79(932):307-12.

14 McAuliffe, L.; Branko K.; Roger D. A.; and Nicholas R. A. (2004): Molecular epidemiological analysis of *Mycoplasma bovis* isolates from the United Kingdom shows two genetically distinct clusters. American Society for Microbiology. Vol. 42, No. 10; 4556-4564.

15 Nicholas, R. A. and Ayling, R. D. (2003): Mycoplasma bovis: disease, diagnosis, and control. Res. Vet. Sci. 74, 105-112.

16 Nicolson, G.L.; Nasralla, M.; Haier, J. and Nicolson, N.L. (1998): Diagnosis and treatment of chronic mycoplasmal infections in fibromyalgia and chronic fatigue syndromes: Relationship to gulf war illness. Biomed. Therapy, 16:266-271.

17 Pfützner, H. and Sachse, K. (1996) *Mycoplasma bovis* as an agent of mastitis pneumonia, arthritis and genital disorders in cattle. Rev. Sci. Tech. Off. Int. Epizoot. , 15:1477–1494.

18 Pfützner, H. (1990): Epizootiology of the *Mycoplasma bovis* infection of cattle. Zentralblatt fur Bakteriologie , 20: 394–399.

19 Postepski, J.; Opoka-Winiarska, V.; Koziol-Montewka, M.; Korobowicz, A. and Tuszkiewicz-Misztal, E. (2003): Role of mycoplasma pneumoniae infection in aetiopathogenesis of juvenile idiopatic arthritis. Med .Wieku. Rozwoj. , 7(2):271-7.

20 Radostits, O.; Blood, D. and Gay, C. (1994): Veterinary Medicine Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses 8th edition. London: Balliere Tindall,;749-758.

21 Rao,V.L. and Murthy,C.R.(1992):Ammonia-induced alteration in the metabolism of glutamate and aspartate in neuronal perikarya and synapatosomes of rat cerebellum.Metab. Brain.Dis. Mar,7(1):51-61.

22 Reitman, S. and Frankel, S. (1957): Colorimetric methods for determination of serum AST, ALT. Amir. J. Clin. Path. 28: 56-58

23 Romváry, J.; Rózsa, J.; Stipkovits, L. and Mészaros, J. (1977): Incidence of diseases due to *Mycoplasma bovis* in a cattle herd. I. Pneumo-arthritis syndrome in calves. Acta Vet. Hung. 27:29-37.

24 Sabry, M. Z. and Ahmed, A. A. (1975): Evaluation of media and cultural procedure for the primary isolation of Mycoplasma from

female genitalia of farm animals. J. Egypt. Vet. Med. Assoc., 35: 18-36.

25 Sonnenwirth, A.C. and Jarett, L. (1980): Gradwohl's Chemical Laboratory Methods and diagnosis 8th Ed. Saintlois C.V. Mosby Co.,

26 Susan J. T. (2006): Mycoplasma haemolamae in camelids: Diagnosis, experimental infections and treatments. Proceedings of the American Association of Veterinary Laboratory Diagnosticians 49TH annual conference October 12 – 18, 2006, p 137

27 Ter Laak, E. A., Noordergraaf, J. H., and Dieltjes, R. P. (1992). Prevalence of mycoplasmas in the respiratory tracts of pneumonic calves. Zentralbl. Veterinarmed. B 39, 553-562.

28 Thomas, L. H.; Howard, C. J.; Stott, E. J. and Parsons, K. R. (1986): *Mycoplasma bovis* infection in gnotobiotic calves and combined infection with respiratory syncytial virus. Vet. Pathol. , 23:571-578

29 Thurmond, M.C.; Holmberg, C.A.and Luiz, D.M. (1989): Evaluation of a Digitonin disc assay to discriminate between *Acholeplasma* and *Mycoplasma* isolates from bovine milk. Cornell Vet., 79: 71-81.

30 UIvund M.J. (1990): Changes in blood chemistry. Acta.Vet. Scand.,31:277-286

31 Walz, P.H.; Mullaney, T.P.and Render, J.A. (1997): Otitis media in preweaned holstein dairy calves in Michigan due to *Mycoplasma bovis*. J. Vet. Diagn. Invest. , 9: 250–254.

32 Wiggins, M.; Woolums, A.; Sanchez, S.; Hurley, D.; Cole, D.; Ensley, D. and Pence, M. (2007): Prevalence of *Mycoplasma bovis* in backgrounding and stocker cattle operations. J. Am. Vet Med. Assoc., 230(10):1514-8.

33 Wise, D.R. and Evans, E. T. (1975): Turkey syndrome 65, oedema syndrome and Mycoplasma meleagridis. Res. Vet. Sci., 18(2):190-2.

34 Yleana, R. Chavez Gonzalez, Carlos Ros Bascunana, Goran Bolske, Jens G. Mattsson, Carmen Fernandez Molina Karl- Erik Johansson. (1995): In vitro amplification of the 16 sRNA genes from *Mycoplasma bovis* and Mycoplasma agalactiae by PCR. Vet. Microbiol., 47: 183-190.

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