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Isolation, Characterization and Antibiogram of *Mycoplasma bovis* in Sheep Pneumonia

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ABSTRACT

M. bovis is a common respiratory pathogen for cattle but it may produce similar disease complex conditions. It can be transmitted through litter, tools and the hands and clothing of the owners and handlers in sheep and goats. In comparisons to other mycoplasmas *M. bovis* shows relatively high resistance under some environmental conditions. The occurrence in sheep is rare but it causes severe economic losses. The present study revealed an outbreak of pneumonia in sheep flock leading to high morbidity and mortality. To get an idea of etiological agent of outbreak the samples were collected from morbid animals and during the postmortem from dead animals. Nasal and tracheal swabs, blood, serum, faecal samples and tissues from multiple organs were collected and examined in laboratory. The *M. bovis* was the etiological agent isolated from nasal swabs and lungs of infected and dead animals, respectively. Histopathological findings also supported mycoplasmic lesions in dead animals. As the success of treatment of the respiratory diseases due to *M. bovis* depends on the right choice of the compound, its distribution in the tissues and last but not least the simultaneous antibacterial effect in secondary and mixed infections, the *in vitro* antimicrobial drug sensitivity was performed to select the drug of choice for treatment. The antibiogram of isolate revealed Tylosin and Enrofloxacin with remarkable zone of inhibition. Therefore, tylosin was recommended as drug for treatment and flock recovered with in the period of 15 days. There was no further mortality and all the infected sheep recovered from the signs of illness. This seems to be the first report of *M. bovis* outbreak in sheep in country.

Key words: *Mycoplasma bovis,* enrofloxacin, pneumonia, sheep, tylosin

INTRODUCTION

Sheep pneumonia is regarded as a complex disease, involving interaction among host, (immunological and physiological), multiple agents, (bacterial, viral, mycoplasma) and environmental factors (Brogden *et al.*, 1998). These can affect any age group of animal but most commonly occurs in lambs aged between 3-12 months when acquired maternal antibody titres are reduced. Cases of chronic pneumonia usually present with ill thrift and varying degrees of

respiratory compromise, with respiratory distress usually evident during exercise (Bell, 2008). Respiratory problems are one of the most common and relevant diseases of sheep in all the sheep-rearing countries. They cause stunted growth, mortality in lambs and important economic impact due to drug costs and condemnations of meat in abattoirs (Jones *et al*., 1982; Goodwin *et al*., 2004). In many countries they play important role in socioeconomics (Ajala *et al*., 2008). Out of many etiological agents Mycoplasma species, particularly *M. bovis*, is harbored in the respiratory tract without showing any apparent clinical symptoms by clinically healthy young cattle (Kumar *et al*., 2011) and shed through their nasal discharge for months or years (Ter Laak *et al*., 1992). *M. bovis* can also colonize sheep (Bocklisch *et al*., 1987) and goat (Egwu *et al*., 2001) and the pathogen can be transmitted among these animals. *M. bovis* shows relative high resistance under some environmental conditions (Nagatomo *et al*., 2001). The success of treatment of *M. bovis* in sheep mainly depends on the choice of the compound used for the treatment, its distribution in the tissues and last but not least the simultaneous antibacterial effect in secondary and mixed infections of e.g. *Pasteurella multocida, Mannheimia haemolytica* or *Haemophilus somnus* (Shayegh *et al*., 2008; Poumarat *et al*., 1994). In this context, present study revealed the involvement of *M. bovis* in sheep pneumonia and the selection of antibiotics for timely recovery of infected animals to prevent economic losses due to mortality and morbidity in sheep flocks.

MATERIALS AND METHODS

Study area, animal and management: The incidence occurred in two flocks of sheep near city Chatta block in district Mathura, located in Uttar Pradesh, India. The sheep population of flocks was 270 at the time of incident. The age of most of the affected animals was between 6 months to two years. The animals were grazed during the day time on natural pasture and had free access to hay (made of natural pasture) at night in properly sheltered pens. Those animals that were kept together during the day time were mostly sheltered at night in same pen. Animals suffering from respiratory disease were supplemented with concentrate feed in separate pen. They had free access to water. All the sheep were drenched against internal parasites with the combination of albendazole and ivermectin approximately one month before the onset of outbreak. Animals were not vaccinated against any disease.

History, clinical examination and data collection: An outbreak with respiratory and nervous signs occurred in the month of August 2010. Infected and suspected animals were thoroughly examined by animal health researchers of Veterinary University, Mathura for pyrexia (rectal temperature more than 105°F), abnormal respiration (sternal and abdominal respiration, polypnoea, dyspnoea), coughing and nasal discharge. Other signs like anorexia, staggering and paresis before death were also recorded. The health and basic epidemiological information viz., morbidity, mortality and case fatality rate were collected and analyzed for occurrence of the disease. Clinical, serological, gross pathological and bacteriological investigations were carried out from infected and post-mortem and histopathological examinations were conducted from dead animals.

Sample collection: Nasal and tracheal swabs, feces, blood and serum samples, were collected from sick animals whereas tissues from spleen, liver, lung, kidney, lymph nodes and hearts were taken from dead animals during post-mortem.

Postmortem and Histopathological examination: Post-mortem examination was conducted immediately after death. During autopsy, special emphasis was given to respiratory organs. Three animals, found dead were subjected to thorough post mortem examination. Necropsy was conducted by the method described by Thompson (1978). Portion of the internal organs of dead lambs (mostly lungs, also spleen, liver, kidney, lymph nodes and heart) were collected for microbiological and histopathological examination aseptically. Standard aseptic procedures were followed for microbial isolation; the surface of the organs were cleaned with denatured alcohol and flamed immediately following the procedures of Carter *et al.* (1995) followed by searing with heated spatula before the inner tissues were chopped and collected in PBS (pH 7.4) and further inoculated into sterile screw capped test tubes containing 5 mL of Tryptose soya/PPLO/SDA broth.

Microbiological culture and identification: Microbiological isolation was performed from nasal swabs and blood samples of sick sheep and internal organs of dead sheep (mostly lungs, also spleen, heart, liver, kidney and lymph nodes). Samples and swabs were inoculated onto nutrient agar, MacConkey Lactose Agar (MLA), Sabouraud's Dextrose Agar (SDA) and 5% sheep blood agar plates and incubated aerobically/microaerobically at 37°C for 24 h (Quinn *et al.*, 2002). After 24 h incubation, the plates were observed for presence of bacterial and fungal colony. Swabs for mycoplasma isolation were homogenized in 1 mL PBS then 100 μL of this solution was transferred into PPLO broth enriched with horse serum under 37°C, 5% CO_2 for 48 h. (Rosengarten *et al.*, 1994) afterwards. Samples from PBS (pH 7.4) were triturated, filter sterilized (0.22 micron syringe filter) and inoculated on MDBK cell lines at 36°C, 5% CO_2 for 48 h in CO_2 incubator.

Lung samples for the isolation of mycoplasma were cultured according to the method described by Ter Laak *et al.* (1992). For mycoplasma slight color change of broth media due to pH shift was examined daily. The cultures were inoculated onto solid Medium using the running drop technique on days 2 and 5 (Lauerman, 1994). The agar plates were incubated at 37°C in 5% CO_2 atmosphere for 14 days. The plates were examined under stereomicroscope and presumptive identification of isolated mycoplasma was made by traditional physiological and biochemical methods (Erno and Stipkovits, 1973a, b).

Serological confirmation of mycoplasma culture: The species-specific identification was performed with anti-*M. bovis* hyperimmune serum which was used in growth inhibition and metabolic inhibition tests (Lauerman, 1994). Identification of *M. bovis* was also performed by DID and slide agglutination test (Bradbury, 1998). Mycoplasma antigen was prepared as per the method described by Kumar (2000).

Antibiogram: Mycoplasma colonies isolated from lung tissues were tested for antimicrobial susceptibility testing by the Bauer *et al.* (1966) following the NCCLS (2002) guidelines. The following commonly used antimicrobial agents (Hi-Media, Mumbai) were used at the indicated concentrations (μg/disc except where specified) viz., Amikacin (30 g), Amoxycillin-clavulanic acid (20/10 μg), Ampicloxacillin (10 μg), Ciprofloxacin (30 μg), Chloramphenicol (30 μg), Enrofolxacin (10 μg), Erythyromycin (15 μg), Levofloxacin (5 μg), Streptomycin (10 μg), Tetracycline (30 μg), Penicillin (10 IU), Amoxicillin (30 μg), Cefotaxime (30 μg), Ceftriaxone (30 μg), Tiamulin (20 μg), Tylosin (20 μg) and Kanamycin (30 μg).

RESULTS AND DISCUSSION

Microbiological examination of nasal swab, blood and tissues of different organs for infectious agent revealed no growth in blood agar, MLA, MDBK cell lines and SDA as there was no bacterial

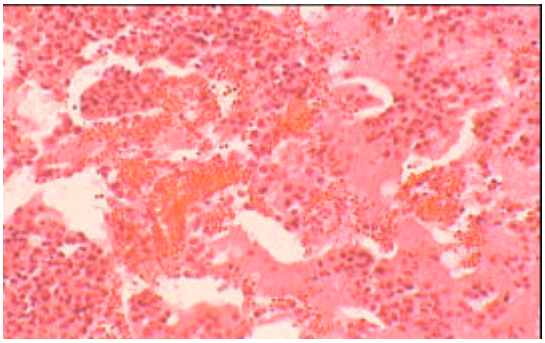

Fig. 1: Edema and haemorrhages

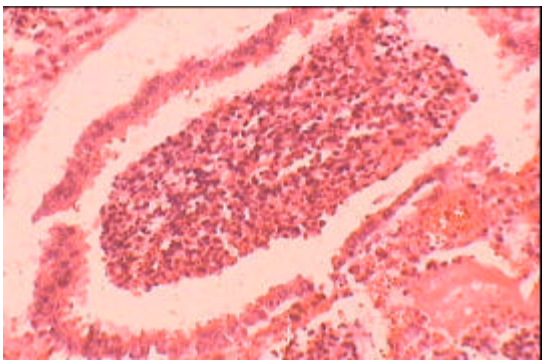

Fig. 2: Exudation and edema in bronchi

growth, cytopathic effects and fungal colonies, respectively. These were suggestive of no involvement of bacteria, virus and fungi. However, PPLO broth revealed change in color without turbidity after the 48 h of incubation. It was suggestive of mycoplasma growth so it was further incubated. After 72 h of incubation PPLO broth was streaked on PPLO agar media and incubated for further examination. After 48 h of incubation it produced small size, circular, convex colonies giving fried egg appearance with central part of colonies darker than periphery. These were confirmed by colony morphology (fried egg appearance colonies), Giemsa staining and digitonin sensitivity test (Kumar, 2000). Slide agglutination test with mycoplasma isolate developed agglutination within one to two minutes. Double immune diffusion with standard sera also produced the line of precipitation after 24 h. These two confirmed the mycoplasma species as *M. bovis*.

Postmortem always help in finding the cause of death (Al-Qudah *et al*., 2008; Almalaik *et al*., 2008). Necropsy showed the presence of both macroscopical and microscopical lung lesions involving the apical and cardiac lobes to the extent of 20-40% of lung area. As *M. bovis* settles down predominantly in the broncho-alveolar region in the respiratory tract the histopathological examination revealed lesions comprised of interstitial pneumonia (Fig. 1) accompanied by perivascular and peribronchial lymphoid cell infiltration (Fig. 2). Accumulation of desquamated cells

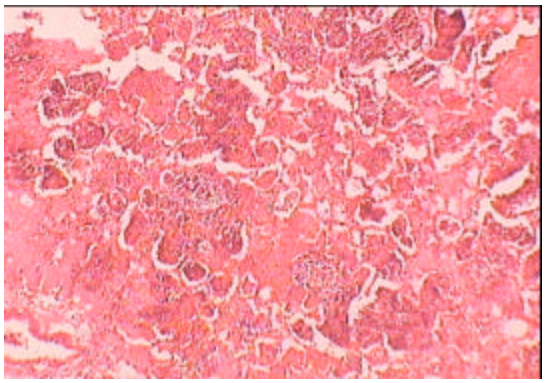

Fig. 3: Congestion in lungs

and serous exudate in bronchioles (Fig. 2) and alveoli with catarrhal pneumonia and the presence of inflammatory foci encapsulated by connective/fibrous tissues (Fig. 3) were also observed. These findings of postmortem were comparable to the findings reported by Rodriguez *et al.* (1996).

M. bovis infection occurs throughout the world (Kumar *et al.*, 2011). It has also been associated with meningitis in calves (Stipkovits *et al.*, 1993), decubital abscesses (Kinde *et al.*, 1993), keratoconjunctivitis (Kirby and Nicholas, 1996; Pfutzner and Sachse, 1996) and otitis media (Walz *et al.*, 1997). In a survey conducted by the Office Internationale d'Epizooties (OIE) in over 48 countries, *M. bovis* was a major component of the calf pneumonia complex, with isolation rates of 23 to 35% (Nicholas *et al.*, 2000). The present study also estabhished *M. bovis* as major and potent cause of morbidity and mortality in two sheep flocks. In Britain alone losses in calves are estimated to be as much as £50 million per year from mortality and costs of treatment (Rebhun *et al.*, 1995).

The outbreak occurred in the end of rainy season during August 2010. Although the sheep were also showing the symptoms of pneumonia but most of the mortality occurred in younger animals and 12 sheep died within the period of one week in spite of continuous treatment. Clinical signs of pneumonia were evident in almost all the animals suffering from disease. The infected animals showed pronounced clinical signs; depression, dyspnoea, respiratory distress, nasal discharge, coughing and pyrexia with progressive emaciation in almost all the cases examined. Few lambs had abundant mucoid to purulent nasal discharge, infrequent lacrimation, scouring or conjuctival congestion and symptoms of pneumonia along with incoordination and inability to rise and move in advance stage ultimately leading to death. As respiratory tract infections in sheep, especially pneumonia continues to be an important cause of economic loss (Tibbo *et al.*, 2001; Al-Qudah *et al.*, 2008). Most of the time respiratory disease is multifactorial in sheep (Lacasta *et al.*, 2008) and a number of causative agents were responsible for the respiratory disease complex including mycoplasma infections. Progress in understanding the pathogenesis of pneumonia has been slow because of its complex etiology and varied epidemiology (Woldemeskel *et al.*, 2002). Therefore, for confirmation of etiological agents gross and histopathological examinations were conducted along with isolation and confirmation of organism.

Further *M. bovis* is considered the mycoplasma of relative high resistance under some environmental conditions (Nagatomo *et al.*, 2001) hence, the role of factors such as the litter, tools and the hands and clothing of the owners and handlers might play role in spreading of the infection. Thus the identification and timely treatment is precious.

The main mode of transmission aerosol route as the infected animals releases a large amount of small, inhaled droplets during coughing. Moreover, infections of *M. bovis* are difficult to treat (Stalheim, 1976) as most *M. bovis* strains are often resistant to antibiotics (Ball *et al*., 1995; Thomas *et al*., 2002). Although, Hannan (2000) recommended that antibiotic susceptibility of the isolates can only be examined by the determination of the Minimal Inhibitory Concentration (MIC) values instead the disc diffusion test but this method can be used for early recommendation of drug of choice and could prevent economic losses. Thus antibiogram of the organism was conducted using the disc diffusion method, *M. bovis* isolates was resistant to almost all the commonly used antibiotics except Tylosin and Enrofloxacin. Ciprofloxacin and Tiamulin showed intermediate response whereas other antibiotics were of no use. Depending upon the zone of inhibition Tylosin was the drug of choice and recommended for the treatment of flock. The findings of present study regarding the drug sensitivity patterns are in the concurrence to the findings of many other workers as according to Ayling *et al*. (2000) and Nicholas *et al*., (2000) all strains of *M. bovis* had developed resistance to tilmicosin, most were found to be resistant to oxytetracycline and about 20% were resistant to spectinomycin and florfenicol. Only danofloxacin was effective *in vitro* against all strains. All of the mycoplasmas are resistant to beta-lactames because of the lack of the cell wall. Poumarat *et al*. (1994) observed resistance against nalidixic acid, polymyxin, rifamycin, trimethoprim and to sulfonamides. Initially tetracyclines, macrohds and aminoglycosides were used with good results against *M. bovis* but later on many resistant strains had been reported (Ball *et al*., 1995; Ayling *et al*., 2000). Similarly the antibiotics of these groups were found resistant and revealed no or very small zone of inhibition. Most of the mycoplasmas including *M. bovis* are sensitive to antibiotics which inhibit the protein or nucleic acid synthesis. The most effective antibiotics are thepleuromutilins (tiamulin, valnemulin) and the fluoroquinolones (Taylor-Robinson and Bebear, 1997; Thomas *et al*., 2003). Tiamulin has been reported with excellent activity against *M. bovis* (Ter Laak *et al*., 1993; Friis and Szancer, 1994; Hannan *et al*., 1997). In contrast to these, the *M. bovis* isolate showed intermediate sensitivity however, similar to the findings of others (Ball *et al*., 1995; Hannan *et al*., 1997; Ayling *et al*., 2000) enrofloxacin was also found effective against the *M. bovis* isolate *in vitro* but some workers do not recommend their use in the practice, because of their failure in diminishing respiratory losses (Nicholas and Ayling, 2003). Therefore, Tylosin was preferred over Enrofloxacin for the treatment of infected flock. The disease conditions induced by *M. bovis* were successfully treated with tylosin. Significant improvement of clinical signs was observed within 3-5 days after the start of medication and clinical signs disappeared within one week. Medication restored the animals' appetite and weight gain approached to the healthy sheep within month and no animal revealed post treatment *M. bovis* in nasal and tracheal swabs.

CONCLUSION

Mycoplasmosis is a common problem in sheep particularly in meditarian countries where it causes severe economic losses. In India there has not been much attention paid to respiratory diseases due to mycoplasma infection. However, many reports of mycoplasma isolation from sheep have been reported from various parts of country time to time. This study appeared to be first of its kind for the isolation of *M. bovis* from the outbreak in sheep flock leading to mortality and high morbidity rate. As mycoplasma differ from cellular composition from bacteria so commonly used antibiotics mostly do not cure them. In this study, tylosin appeared to be the drug of choice and its application cured the flock.

REFERENCES

Ajala, M.K., O.S. Lamidi and S.M. Otaru, 2008. Peri-Urban small ruminant production in Northern Guinea Savanna, Nigeria. AJAVA., 3: 138-146.

Al-Qudah, K.M., A.M. Al-Majali and M.M. Obaidat, 2008. A study on pathological and microbiological conditions in goats in slaughterhouses in Jordan. Asian J. Animal Vet. Adv., 3: 269-274.

Almalaik, A.H.A., A.E. Bashar and A.D. Abakar, 2008. Prevalence and dynamics of some gastrointestinal parasites of sheep and goats in tulus area based on post-mortem examination. Asian J. Anim. Vet. Adv., 3: 390-399.

Ayling, R.D., S.E. Baker, R.A.J. Nicholas, M.L. Peek and A.J. Simon, 2000. Comparison of *in vitro* activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against recent field isolates of *Mycoplasma bovis*. Vet. Rec., 146: 745-747.

Ball, H.J., C. Reilly and D.G. Bryson, 1995. Antibiotic susceptibility of *Mycoplasma bovis* strains isolated in Northern Ireland. Irish Vet. J., 48: 316-318.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bell, S., 2008. Respiratory diseases of sheep: Differential diagnosis and epidemiology. Practice, 300: 200-207.

Bocklisch, H., V. Zepezauer, H. Pfutzner and S. Kreusel, 1987. Demonstration of mycoplasma in sheep pneumonia and experimental pneumonia produced by *Mycoplasma arginini*. Arch. Exp. Veterinarmed., 41: 249-257.

Bradbury, J.M., 1998. Identification of Mycoplasmas by Immunofluorescence. In: Methods in Molecular Biology, Miles, R. and R. Nicholas (Eds.). Vol. 104. Humana Press, Totowa, NJ., USA., pp: 119-125.

Brogden, K.A., H.D. Lehmkuhi and R.C. Cutlip, 1998. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. Vet. Res., 29: 233-254.

Carter, G.R., M.M. Chengappa and A.W. Roberts, 1995. Essentials of Veterinary Microbiology. 5th Edn., Williams and Wilkins, Baltimore, MA., USA., ISBN-13: 9780683014730, Pages: 394.

Egwu, G.O., J.A. Ameh, M.M. Aliyu and F.D. Mohammed, 2001. Caprine mycoplasmal mastitis in Nigeria. Small Rumin. Res., 39: 87-91.

Erno, H. and L. Stipkovits, 1973a. Bovine mycoplasmas: Cultural and biochemical studies. I. Acta Vet. Scand., 14: 436-449.

Erno, H. and L. Stipkovits, 1973b. Bovine mycoplasmas: Cultural and biochemical studies. II. Acta Vet. Scand., 14: 450-463.

Friis, N.F. and J. Szancer, 1994. Sensitivity of certain porcine and bovine mycoplasmas to antimicrobial agents in a liquid medium test compared to a disc assay. Acta Vet. Scand., 35: 389-394.

Goodwin, K.A., R. Jackson, C. Brown, P.R. Davies, R.S. Morris and N.R. Perkins, 2004. Pneumonic lesions in lambs in New Zealand: Patterns of prevalence and effects on production. N. Z. Vet. J., 52: 175-179.

Hannan, P.C.T., G.D. Windsor, A. Jong, N. Schmeer and M. Stegemann, 1997. Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. Antimicrob. Agents Chemother., 41: 2037-2040.

Hannan, P.C., 2000. Guidelines and recommendations for antimicrobial Minimum Inhibitory Concentration (MIC) testing against veterinary mycoplasma species. International research programme on comparative mycoplasmology. Vet. Res., 31: 373-395.

Jones, G.E., A.C. Field, J.S. Gilmour, A.G. Rae, P.F. Nettleton and M. McLauchlan, 1982. Effects of experimental chronic pneumonia on body weight, feed intake and carcase composition of lambs. Vet. Rec., 110: 168-173.

Kinde, H., B.M. Daft, R.L. Walker, B.R. Charlton and R. Petty, 1993. *Mycoplasma bovis* associated with decubital abscesses in Holstein calves. J. Vet. Diagn Invest., 5: 194-197.

Kirby, F.D. and R.A. Nicholas, 1996. Isolation of *Mycoplasma bovis* from bullocks eyes. Vet. Rec., 138: 552-552.

Kumar, A., 2000. Characterization of protein antigens of *Mycoplasma agalactiae*. M.Sc. Thesis, IVRI, Izatnagar, UP. India.

Kumar, A., A.K. Verma and A. Rahal, 2011. *Mycoplasma bovis*, a multi disease producing pathogen: An overview. Asian J. Anim. Vet. Adv., 6: 537-546.

Lacasta, D., L.M. Ferrerb, J.J. Ramosb, J.M. Gonzaleza and M. De las Heras, 2008. Influence of climatic factors on the development of pneumonia in lambs. Small Ruminant Res., 80: 28-32.

Lauerman, L.H., 1994. Mycoplasmas of the Bovine Respiratory Tract. In: Mycoplasmosis in Animals: Laboratory Diagnosis, Whitford, H.W., R.F. Rosenbusch and L.H. Lauerman (Eds.). Iowa State University Press, Ames, Iowa, pp: 50-56.

NCCLS, 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard M31-A2. 2nd Edn., National Committee for Clinical Laboratory Standards, Wayne, PA.

Nagatomo, H., Y. Takegahara, T. Sonoda, A. Yamaguchi, R. Uemura, S. Hagiwara and M. Sueyoshi, 2001. Comparative studies of the persistence of animal mycoplasmas under different environmental conditions. Vet. Microbiol., 82: 223-232.

Nicholas, R.A., S.E. Baker, R.D. Ayling and L. Stipkovits, 2000. Mycoplasma infection in growing cattle. Cattle Pract., 8: 115-118.

Nicholas, R.A. and R.D. Ayling, 2003. *Mycoplasma bovis*: Disease, diagnosis and control. Res. Vet. Sci., 74: 105-112.

Pfutzner, H. and K. Sachse, 1996. Mycoplasma bovis as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. Rev. Sci. Tech., 15: 1477-1494.

Poumarat, F., M. Solsona and M. Boldini, 1994. Genomic, protein and antigenic variability of *Mycoplasma bovis*. Vet. Microbiol., 40: 305-321.

Quinn, P.J., B.K. Markey, M.E. Carter, W.J.C. Donnelly and F.C. Leonard, 2002. Veterinary Microbiology and Bacterial Disease. Blackwell Science, London, UK., Pages: 648.

Rebhun, W.C., C. Guard and C.M. Richards, 1995. Respiratory Diseases. In: Diseases of Dairy Cattle, Divers, T.J. and S.F. Peek (Eds.). Williams and Wilkins, Baltimore, MA., USA., pp: 79-80.

Rodriguez, F., D.G. Bryson, H.J. Ball and F. Forster, 1996. Pathological and immunohistochemical studies of natural and experimental *Mycoplasma bovis* pneumonia in calves. J. Comp. Pathol., 115: 151-162.

Rosengarten, R., A. Behrens, A. Stetefeld, M. Heller and M. Ahrens *et al*., 1994. Antigen heterogeneity among isolates of *Mycoplasma bovis* is generated by high-frequency variation of diverse membrane surface proteins. Infect. Immun., 62: 5066-5074.

Shayegh, J., S. Atashpaz and M.S. Hejazi, 2008. Virulence genes profile and typing of ovine *Pasteurella multocida*. Asian J. Anim. Vet. Adv., 3: 206-213.

Stalheim, O.H., 1976. Failure of antibiotic therapy in calves with mycoplasmal arthritis and pneumonia. J. Am. Vet. Med. Assoc., 169: 1096-1097.

Stipkovits, L. M. Rady and R. Glavits, 1993. Mycoplasmal arthritis and meningitis in calves. Acta Vet. Hung., 41: 73-88.

Taylor-Robinson, D. and C. Bebear, 1997. Antibiotic susceptibilities of mycoplasmas and treatment of mycoplasmal infections. J. Antimicrob. Chemother., 40: 622-630.

Ter Laak, E.A., G.H. Wentink and G.M. Zimmer, 1992. Increased prevalence of *Mycoplasma bovis* in the Netherlands. Vet. Q., 14: 100-104.

Ter Laak, E.A., J.H. Noordergraaf and M.H. Verschure, 1993. Susceptibilities of *Mycoplasma bovis, Mycoplasma dispar* and *Ureaplasma diversum* strains to antimicrobial agents *in vitro*. Antimicrob. Agents Chemother., 37: 317-321.

Thomas, A., I. Dizier, A. Trolin, J. Mainil and A. Linden, 2002. Comparison of sampling procedures for isolating pulmonary mycoplasmas in cattle. Vet. Res. Commun., 26: 333-339.

Thomas, A., C. Nicolas, I. Dizier, J. Mainil and A. Linden, 2003. Antibiotic susceptibilities of recent isolates of *Mycoplasma bovis* in Belgium. Vet. Rec., 153: 428-431.

Thompson, R.G., 1978. General Veterinary Pathology. W.B. Saunders, Philadelhia, pp: 412-413.

Tibbo, M., M. Woldemeskel and A. Gopilo, 2001. An outbreak of respiratory disease complex in sheep in Central Ethiopia. Trop. Anim. Health Prod., 33: 355-365.

Walz, P.H., T.P. Mullaney, J.A. Render, R.D. Walker, T. Mosser and J.C. Baker, 1997. Otitis media in preweaned Holstein dairy calves in Michigan due to *Mycoplasma bovis*. J. Vet. Diagn Invest., 9: 250-254.

Woldemeskel, M., M. Tibbo and L.N. Potgieter, 2002. Ovine progressive pneumonia (Maedi-Visna): An emerging respiratory disease of sheep in Ethiopia. Dtsch. Tierarztl. Wochenschr., 109: 486-488.