Applied Biological Research 17(3): 311-314; (2015)
DOI: 10.5958/0974-4517.2015.00045.2

SHORT COMMUNICATION

MOLECULAR DETECTION OF *Mycoplasma* spp. ASSOCIATED WITH THE FEMALE REPRODUCTIVE TRACT OF CATTLE AND BUFFALOES

Paviter Kaur*, N.S. Sharma, A.K. Arora and T.S. Rai

Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana - 141 004, Punjab (India)
*e-mail: *paviterkaur17@gmail.com*

(Received 8 June, 2015; accepted 15 October, 2015)

ABSTRACT

The present study was aimed to detect *Mycoplasma* spp. associated with the female reproductive tract of cattle and buffaloes in Punjab (India). Out of 65 samples comprising of uterine discharges, vaginal mucus and foetal stomach contents, 21 were positive for *Mycoplasma* spp. by genus specific PCR using primers based on 16S rDNA of *Mycoplasma* spp. An amplification band of 270-bp was obtained in all the positive samples. The overall prevalence of *Mycoplasma* spp. in cattle and buffaloes was 32.3% whereas the prevalence was 24.61 and 7.69% in cattle and buffaloes, respectively. Maximum prevalence of 21.7% was seen in HF breed of cattle and 15.78% in Murrah breed of buffaloes. Cattle and buffaloes in age group 4-6 years and 6-8 years were found more positive for *Mycoplasma* spp.

Key words: Buffaloes, cattle, *Mycoplasma*, PCR, uterine discharges, vaginal mucus

INTRODUCTION

Mycoplasma are the smallest, self-replicating organisms devoid of a cell wall (Amin *et al*., 2013) and have the ability to colonize reproductive tract of bovines (Marouf *et al*., 2011) leading to reproductive disorders and abortions (Yassin *et al*., 2012). In addition to causing sporadic abortions, *Mycoplasma* spp. reportedly cause diseases such as vaginitis, cervicitis, endometritis, salpingitis and infertility. Infection of female reproductive tract may be acquired either from the contaminated environment or much more from artificial insemination with contaminated semen (Pfutzner and Sachse, 1996) whereas infection in males can occur through contact with other animals or possibly through a heavily contaminated environment (Marouf *et al*., 2011). Diagnosis can be achieved by serological methods or by isolation of the organism. While serological procedures suffer from the problem of cross-reactivity, isolation of *Mycoplasma* is time-consuming, difficult and hard to achieve for some fastidious *Mycoplasma* (Yassin *et al*., 2012). Molecular methods, such as polymerase chain reaction (PCR), surpass these drawbacks and have been carried out by various researchers (Cai *et al*., 2005; Chaudhary *et al*., 2013; Naher and Said, 2013; Metwally *et al*., 2014; Qasem *et al*., 2015). Very few reports are available on studies about the involvement of *Mycoplasma* in reproductive tract infections of cattle and buffaloes. Since isolation of *Mycoplasma* is very difficult, therefore, the present study was aimed to employ PCR for rapid detection of *Mycoplasma* spp. associated with the female reproductive tract of cattle and buffaloes.

MATERIALS AND METHODS

A total of 65 samples comprising of uterine discharges (31), vaginal mucus (25) and foetal stomach contents (9) were collected from cattle (46) and buffaloes (19) suffering from reproductive disorders and abortions from various regions of Punjab in 2014. The animals were divided into four groups according to age (0-2, 2-4, 4-6 and 6-8 years). Breed-wise prevalence was studied in Holstein Friesian, indigenous and cross bred breeds of cows and in Murrah and mixed breeds of buffaloes. DNA was extracted from the samples by using Hipure genomic DNA purification kit (Himedia Mumbai) following the manufacturer's instructions. DNA of *Mycoplasma* spp. used as a positive control was obtained from IVRI, Izatnagar (India). For PCR, *Mycoplasma* genus specific primers based on 16S rDNA were used as per Marois *et al*. (2000). Primer sequence comprised of 5'-TGCACCATCTGTCACTCTGTTAACCTC-3' (f) and 5'-GGGAGCAAACAGGATTAGATA CCCT-3' (r). PCR was carried out in a reaction volume containing ReddyMix PCR master mix (Thermo Scientific), 20 pmol of each primer, 5 µL template DNA and nuclease free water to make volume upto 25 µL. Cycling parameters consisted of initial denaturation at 90°C for 1 min, followed by 40 cycles each of 95°C for 15 sec. (denaturation), 58°C for 20 sec. (annealing) and 75°C for 20 sec. (extension), and a final cycle of denaturation at 95°C for 15 sec.; annealing at 58°C for 45 sec. and final extension at 72°C for 10 min. PCR amplified products were separated using electrophoresis in 1.0% agarose gel containing ethidium bromide for 1.5 hr at 80V and visualized under gel documentation system.

RESULTS AND DISCUSSION

Twenty one (21) samples out of the 65 samples were found positive for *Mycoplasma* spp. and an amplification band of 270-bp. was obtained in all the positive samples (Fig. 1). The overall prevalence of *Mycoplasma* spp. in cattle and buffaloes was 32.3% whereas the prevalence was 24.6 and 7.69% in cattle and buffaloes, respectively. The prevalence of *Mycoplasma* spp. in samples from different breeds of cattle and buffaloes revealed maximum prevalence of 21.73% in HF breed of cattle and 15.78% in Murrah buffaloe. Earlier studies have shown the presence of *Mycoplasma* in genital tracts of healthy and diseased cows (Panangala *et al.*, 1978; Kirkbride, 1987; Buzinhani *et al.*, 2007; Petit *et al.*, 2008).

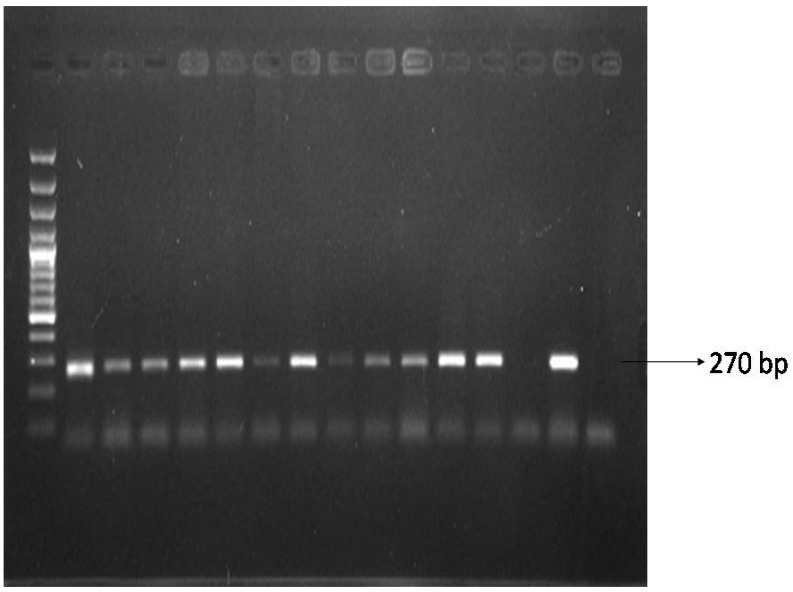

Fig. 1: Gel electrophoresis of PCR amplified fragments of *Mycoplasma* species [Lane 1: 100 bp DNA ladder, Lane 2 to 13: positive samples, Lane 14: negative sample, Lane 15: positive control, and Lane 16: negative control]

Mycoplasma cause granular vulvo-vaginitis and damage the inner lining of the oviduct in females. *M. bovis* is capable of causing subacute to acute inflammation of genital tracts in young and adult cattle (Pfutzner and Sachse, 1996) whereas *M. bovigenitalium* and *M. agalactiae* are frequently associated with seminal vesiculitis in male and metritis and salpingitis in female (Perumal *et al.*, 2013). The presence of *M. bovigenitalium* in 43% males and in cervico-vaginal mucus of 47% females [25% foetuses and 11% placenta] in cattle has been reported (Trichard and Jacobsz, 1985). An incidence of 2.2 and 10% for *M. bovis* and 13.3 and 10% for *M. bovigenitalium* has been reported from vaginal swabs of infertile cows and buffaloes by Marouf *et al.* (2011). A total of 1.18% foetal stomach contents of aborted bovine fetuses were positive for *M. bovoculi* (Singh *et al.*, 2004). Mohammed *et al.* (2013) detected *M. bovigenitalium* in the uterus of postpartum cows with an incidence of 7.4% and suggested that it might be associated with cytological endometritis in postpartum dairy cows. The differences in the incidence of *Mycoplasma* in present study in comparison to the previous reports could be due to variation in animal age and breed and the type of sample taken. In the present study, cattle and buffaloes in age group of 4-6 and 6-8 years were found maximum positive for *Mycoplasma* spp. No proper studies have so far been reported which corroborate the relationship of age of animal, breed and type of sample. to the presence of *Mycoplasma*. However, Edward and Fitzgerald (1952) reported their inability to recover *Mycoplasma* from vagina of maiden heifers. Langford (1975) reported an overall incidence of 21% in one and two year old heifers with incidence in 2 year old appearing to be higher than in one year old. This thereby indicates that the incidence of *Mycoplasma* at different ages is related to the number of times the animal has been bred. In conclusion, prevalence of *Mycoplasma* spp. associated with female reproductive tract of cattle and buffaloes vary with the type of sample collected, the age and the breed of the animals.

Acknowledgements: Facilities provided by GADVASU, Ludhiana, Punjab (India) to carry out the present investigation is duly acknowledged.

REFERENCES

Amin, S., Rahman, S.U., Hussain, I. and Muhammad, G. 2013. Seroprevalence of Mycoplasma ovipneumoniae among sheep from different districts of Baluchistan, Pakistan. *International Journal of Agriculture and Biology,* **15:** 1043-1046.

Buzinhani, M., Metiffogo, E. and Timenetsky, J. 2007. Detection of *Mycoplasma* spp. and *Ureaplasma diversum* in cows with reproductive disorders. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia,* **59**(6): [*http://dx.doi.org/10.1590/S0102-09352007000600003*].

Cai, H.Y., Bell-Rogers, P., Parker, L. and Prescott, J.F. 2005. Development of a real-time PCR for detection of *Mycoplasma bovis* in bovine milk and lung samples. *Journal of Veterinary Diagnostic Investigation,* **17:** 537-545.

Chaudhary, R., Sutikshan, S., Sabah, J., Kapil P., Dey, A.B. and Malhotra P. 2013. Molecular detection of *Mycoplasma pneumoniae* by quantitative real-time PCR in patients with community acquired pneumonia. *Indian Journal of Medical Research,* **138:** 244-251.

Edward, D.G.F.F. and Fitzgerald, W.A. 1952. A growth factor needed to isolate organisms of the pleuropneumonia group from the genital tract of cattle. *Veterinary Record,* **64:** 395.

Kirkbride, C.A. 1987. Mycoplasma, Ureaplasma and Acholeplasma infections of bovine genitalia. *Veterinary Clinics of North America: Food Animal Practice,* **3**: 575-591.

Langford, E.V. 1975. *Mycoplasma* species recovered from the reproductive tracts of Western Canadian cows. *Canadian Journal of Comparative Medicine,* **39:** 133-138.

Marois, C., Dufour-Gesbert, F. and Kempf, I. 2000. Detection of *Mycoplasma synoviae* in poultry environment samples by culture and polymerase chain reaction. *Veterinary Microbiology,* **73**: 311-318.

Marouf, S.A., Mohamed, K.F. and Jakea, J.El. 2011. Detection of *Mycoplasma bovis* and *M.bovigenitalium* in cattle and buffaloes in Egypt using Dot-ELISA and PCR with anti microbial trials. *European Journal of Biological Sciences,* **3**: 1-8.

Metwally, M.A., Yassin, A.S., Essam, T.M., Hamouda, H.M. and Amin, M.A. 2014. Detection, characterisation, and molecular typing of human *Mycoplasma* spp. from major hospitals in Cairo, Egypt. *Scientific World Journal,* [*http://dx.doi.org/10.1155/2014/549858*].

Mohammed, E.G., Hidetoshi, H., Erisa, T., Hideki, I., Bhuminand, D., Yoshiaki, I. and Takeshi O. 2013. Mycoplasma infection in the uterus of early postpartum dairy cows and its relation to dystocia and endometritis. *Theriogenology,* **79:** 180-185.

Naher, H.S. and Said I.H. 2013. Culturing and PCR methods for detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in women with genitourinary tract infections. *International Research Journal of Medical Sciences,* **1(3):** 25-29.

Nakamura, R.M., Waltc, M.L. and Bennettd, R.H. 1977. Studies of bovine genital tracts: mycoplasma isolations from dairy cows. *Theriogenology,* **7:** 351–355.

Panangala, V.S., Fish, N.A. and Barnum, D.A. 1978. Microflora of the cervico-vaginal mucus of repeat breeder cows. *Canadian Veterinary Journal,* **19:** 83–89.

Perumal, P., Kiran,. K.T. and Srivastava, S.K. 2013. Infectious causes of infertility in buffalo bull (*Bubalus bubalis*). *Buffalo Bulletin,* **32(2):** 71-96.

Petit T., Spergser J., Aurich J. and Rosengarten R. 2008. Examination of semen from bulls at five Austrian artificial insemination centres for chlamydiae and mollicutes. *Veterinary Record,* **162:** 792–793.

Petit, T., Spergser, J., Aurich, J. and Rosengarten, R. 2008. Prevalence of Chlamydiaceae and Mollicutes on the genital mucosa and serological findings in dairy cattle. *Veterinary Microbiology*, **27:** 325–333.

Pfutzner, H. and Sachse, K. 1996. *Mycoplasma bovis* as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. *Revue Scientifque Et Technique De L Office International Des Epizooties.* **15**: 1477-1494.

Qasem, J.A., Mouqati, S.A.A., Ali, E.M.A. and Haji, A.B. 2015. Application of molecular and serological methods for rapid detection of *Mycoplasma gallisepticum* infection (avian mycoplasmosis). *Pakistan Journal of Biological Sciences,* **18:** 281-287.

Singh, Y., Garg, D.N. and Kapoor, P.K. 2004. Isolation of *Mycoplasma bovoculi* from genitally diseasesd bovines and its experimental pathogenicity in experimental guinea pigs. *Indian Journal of Experimental Biology*, **42:** 933-936.

Trichard, C.J. and Jacobsz, E.P. 1985. Mycoplasmas recovered from bovine genitalia, aborted foetuses and placentas in the Republic of South Africa. *Onderstepoort Journal of Veterinary Research,* **52:** 105–110.

Yassin, M.H., Al-Humianay, A.A. and Mansour, A.M.A. 2012. Colistin sulphate as a substitute of thallium acetate in culture media for isolation of *Mycoplasma* from cattle diseased by Mycoplasmosis. *International Journal of Microbiology Research,* **3:** 128-132.