



Molecular identification and capsular typing of *Pasteurella multocida* isolates from sheep pneumonia in Iran

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ABSTRACT

Pasteurella multocida is known as one of the main organisms causing pneumonia in sheep. As immunity in pasteurellosis is serogroup specific, identification of prevalent capsular group among endemic areas is essential. The aim of this study was to molecular identification and determine of the capsular type of the *P. multocida* strains isolated from sheep pneumonia in Iran. Bacteriological and biochemical characterization was confirmed by species specific PM-PCR. Genomic DNA was extracted by boiling method. Capsular typing was conducted by using type specific primers via Cap-PCR method. The PCR product was sequenced and analysed by BLAST software. The biochemical identification was confirmed by a species-specific PCR assay (PM-PCR). According to Cap-PCR results, all of the 52 *P. multocida* isolates belonged to the capsular type A. The PCR amplified a fragment of 1044 bp from all of tested isolates. The sequence alignment of the CAP-PCR product showed a high similarity (>98%) with the published sequences of *P. multocida* hya gene in the Gene Bank. It was found that capsular type A is dominant among *P. multocida* isolates from sheep pasteurellosis in endemic areas of Iran. Investigation on preparation and evaluation of an effective sheep pasteurellosis vaccine by using ovine isolates of *P. multocida* capsular type A is recommended.

1. Introduction

Pasteurella multocida is a gram-negative bacteria pathogen associated with a variety of diseases in animals, causing haemorrhagic septicaemia in cattle and buffaloes, fowl cholera in poultry, atrophic rhinitis in pigs, snuffles in rabbits, pneumonic and septicaemic pasteurellosis in sheep, goats, wild animals and humans (De Alwis, 1996). It has been considered as a highly variable pathogen due to the diversity observed among serotypes with respect to host predilection, pathogenicity, biochemical, cultural and antigenic specificity

(Carter and Chengappa, 1981). The detection and identification of *P. multocida* on the basis of cultural and antigenic methods is well established (Carter, 1975, Heddleston et al., 1972).

A capsulated strain of *P. multocida* can be separated in one of the serological groups A, B, D, E and F based on the differences in the capsular polysaccharides. It is known that besides the geographical distribution, these sero-groups are more or less specific with regard to the hosts and the diseases induced (Boyce et al., 2000).

Serogroups A and D are world-wide spread serogroups which can be found in a wide range of

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domestic animals (e.g. from fowl to calves, pigs, sheep, goats, and rabbits) in which they cause various infections (Confer, 1993; Quinn *et al.*, 1994). Serogroups B and E have been found predominantly in tropical areas where they induce haemorrhagic septicemia in cattle and wild ruminants (Carter and Chengappa, 1991; Townsend *et al.*, 1997). Capsule serogroup F was first isolated from turkeys in the U.S.A. (Rimler and Rhoades, 1987), and isolates of this serogroup have usually originated from birds situated in North America (Rhoades and Rimler, 1987; Wilson *et al.*, 1993; Rimler, 1994; Wilson *et al.*, 1995) and less frequently in other parts of the world. The serogroup F has been known as a causative agent of fowl cholera (Rimler and Rhoades, 1987) but, in recent time, it has also been found in some mammalian species in deferent world areas (Davies *et al.*, 2003).

It was demonstrated that the major polysaccharide component of the capsule in serogroup A is hyaluronic acid (Rosner *et al.*, 1992). On the basis of decapsulation profiles of *P.multocida* by action of mucopolysaccharidases, it was proposed that serogroups D and F produced capsular materials that contained heparin and chondroitin-sulfates, respectively.

The capsules of several bacteria including *P.multocida* are important virulence factors (Chung *et al.*, 1998; Boyce and Adler, 2000) which plays vital roles in the pathogenicity of pathogenic bacteria and establishment of infection. The virulence mechanism of the cell capsule is mostly attributed to its ability to protect the invading organism against cellular and humoral defense mechanisms of the host. The capsular materials of *Pasteurella* species were identified as polysaccharide basic structures produced during the logarithmic phase of bacterial growth. Each serotype of *P.multocida* produces a characteristic polysaccharide capsule in order to avoid phagocytosis by macrophages and polymorphonuclear leukocytes and to protect the organism against complement-mediated destruction of the outer membrane in serum (Brogden *et al.*, 1989; Mohamed and Abdelsalam., 2008).

Conventional methods of characterizing isolates of *P.multocida* are based on capsular and somatic serotyping, which are often time-consuming and do not type all strains (Rimler *et al.*, 1998).

The main serological method for capsular typing of *P.multocida* based on detection of specific capsular antigens is indirect haemagglutination test (IHA) as described by Carter (1955). It was found that production of specific sera against capsular antigens especially serogroups A, D and F are difficult.

The entire capsule biosynthetic locus of *P.multocida* has been sequenced and the components responsible for the synthesis of capsular antigens were identified (Chung *et al.*, 1998). Based on these findings, a capsular PCR typing system has been developed and shown to provide a rapid, sensitive and highly type-specific identification method. This assay represented a suitable and reproducible alternative instead of the more complicated serological and non-serological typing methods (Townsend *et al.*, 2001). The objective of the presented study was to identify the capsular type (s) of ovine *P.multocida* isolates obtained from sheep pneumonia in endemic provinces of Iran by PCR assay.

2. Materials and Methods

2.1. Bacteria

The nasal and tonsil swab samples were collected from ailing sheep. The ailing animals exhibited pneumonic symptoms, respiratory distress, profuse nasal discharge, and sneezing. The swab samples were then transferred on dry ice to the laboratory.

A total of 52 field isolates of *P.multocida* from sheep with respiratory diseases from endemic areas in Fars (15), Isfahan (14), Ghom (18) and Tehran (5) provinces were included in this study. The reference strains of *P.multocida* used in the study were PMI30 (capsular serogroup A) and PMI25 (capsular serogroup B) obtained from Aerobic Bacterial Vaccines Dept, Razi Inst. Iran.

2.2. Isolation of *Pasteurella multocida*

Specimens such as nasal and oropharyngeal swabs were inoculated into 2 ml of brain heart infusion broth and incubated at 37°C for 18 hours. A pair of mice was inoculated subcutaneously with 0.2 ml of the broth material. Heart blood were aspirated from the dead mice using sterile Pasteur pipette, observing all aseptic precautions and the aspirated heart bloods were streaked directly on to

blood agar and incubated at 37°C for 24-48h. The colonies suggestive of *P.multocida* were subjected to gram staining, catalase and oxidase test and growth on MacConkey agar for identification.

The cultural, morphological and biochemical tests were carried out according to standard procedures. Carbohydrate fermentation test was conducted to identify the ability of the isolates to catalyze arabinose, dextrin, dulcitol, galactose, glycerol, inositol, inulin, lactose, maltose, manitol, raffinose, sorbitol, sucrose, trehalose, and xylose (Quinn *et al.*, 1994).

2.3. Pathogenicity test

Pathogenicity of each isolate was tested in six weeks Balb/c mice. Each group of mice (three mice in each group) was inoculated intra-peritoneally with 0.1 ml of culture containing 0.3×10^8 organisms per ml. A control group of mice was injected with a fresh BHI broth. All the mice were kept under observation for 72 h and mortality was recorded. Blood smears were prepared from the heart blood of dead mice and stained with Giemsa stain. Liver and lung samples from the dead mice were streaked onto 10% sheep blood agar and incubated at 37°C for 24 h. Aspirated heart blood samples were inoculated in BHI broth as well and incubated at 37°C for 18 h, and the broth cultures were streaked onto blood agar and MacConkey agar.

2.4. DNA extraction

Genomic DNA of all the isolates used in this study were extracted by the method of Wilson *et al.* (1995) with a little modifications (Jabbari *et al.*, 2002).

The cultures were initially grown on 5% sheep blood agar plates with incubation at 37°C for 18 to 24 hours. Cultured bacteria from a single colony was inoculated into a test tube containing 3 ml brain heart infusion broth (BHI) and incubated at 37°C for 18-24 hours with a little rotation to get approximately 10^8 cells/ml.

1.5 ml aliquots of the BHI were centrifuged at 13000 x g for 5 minutes. The supernatant were discarded and the pellets were washed twice in PBS at 13000x g for five minutes. The bacterial cell pellets were resuspended in 600 µl natrium tris EDTA (NTE) buffer pH 7.4. Three µl of a 20

mg/ml stock solution of proteinase K (Gibco/BRL) was added to the bacterial suspensions to a final concentration of 100 µg/ml. The bacterial suspensions were then incubated at 37°C overnight in the presence of 0.5% SDS. The cell lysate was extracted with one volume of ultra pure phenol (Gibco/BRL) previously saturated with Tris pH 8.3. The mixture was rotated vigorously for 5 minutes at room temperature and then centrifuged at 13000x g for 10 minutes. The upper phase was collected without disrupting the interface and transferred to a clean 1.5 ml microtube. This phase was mixed with one volume of a phenol/chloroform /isoamylalcohol (24: 24: 1) mixture and centrifuged at 13000 x g for 5 minutes. The extraction with phenol/ chloroform/ isoamylalcohol was repeated until there was no visible material at the interface between the aqueous and organic phase.

The aqueous DNA solution was then precipitated by the addition of 1/10 volume of 3M sodium acetate pH 4.8, two volumes of cold absolute ethanol and mixed gently by swirling the tube. The precipitated nucleic acid was removed by washing it out by a hooked pipette. The DNA was then washed with 70% ethanol, dried at room temperature, resuspended in 200 µl of TE buffer pH 8.0 and kept at 4°C overnight. The DNA was stored at -20°C until required.

The absorbance of DNA was measured at 260 nm in a spectrophotometer (Pharmacia, ultraspec, 2000). The concentration of DNA was estimated assuming that one OD unit at 260 nm corresponded to 50 µg/ ml of DNA (Sambrook *et al.*, 1989).

PCR was also performed from direct colony and bacterial lysates. An overnight cell culture grown in Brain Heart Infusion (BHI) broth was harvested by centrifugation at 11,337 x g for 5 min. The cell pellet was resuspended in 100 µl of TE (10 mM Tris; 1 mM EDTA, pH 8.0) buffer and boiled for 10 min, followed by immediate chilling. The cell lysate was centrifuged at 11,337 x g for 5 min and the supernatant was used as the DNA template. The concentration of the DNA template was determined using a spectrophotometer at OD_{260/280nm} (Eppendorf, Germany).

2.5. Primers

The isolates were initially checked with *P.multocida* specific PCR (PM-PCR) using primers, KMT1T7 and KM T1SP6. The forward and reverse

primers were 5'-ATC CGC GAT TTA CCC AGT GG-3' and 5'- GCT GTA AAC GAA CTC GCC AC-3', respectively. The amplified fragment size of PCR product was 460 bp. The *P.multocida* capsular serogroup A and B specific primers designed by Townsend *et al.* (2001) were used. The forward primer, 5'-TGC CAA AAT CGC AGT CAG-3' and the reverse primer, 5'-TTG CCA TCA TTG TCA GTG-3' are specific for serogroup A. The forward and reverse primers was 5'-CAT TTA TCC AAG CTC CAC C-3' and 5'-GCC CGA GAG TTT CAA TCC-3', respectively which are specific for serogroup B. The amplicon size for serogroup A and serogroup B specific primers are 1044 bp and 760 bp, respectively. The reference strains of *P.multocida* CAPA (PMI30) and CAPB (PMI35) from Razi Institute, Iran were used for standardization of PCR.

2.6. PCR conditions

Capsular typing was done via Polymerase Chain Reaction (PCR). The detection of capsular genes by PCR for all serogroups were done according to the method described by Townsend *et al.* (2001) with a little modifications. Each reaction mixture contained 50 ng of DNA template, 1 X PCR Buffer, 280 μ M dNTPs mix, 2.7 mM MgCl₂ buffer and 1.5 U of Taq DNA polymerase (Promega, USA). The primer concentrations used were: primers KMT1T7, KMT1SP6 (*P.multocida*) and A- FWD, A-REV (serogroup A) 3.2 μ M and primers B-FWD, B-REV (serogroup B) 1 μ M. (Operon, Germany).

PCR amplifications were prepared with a mixture (50 μ l) containing each primer at a concentration of 3.2 μ M, each deoxynucleoside triphosphate at a concentration of 200 μ M, 1x PCR buffer, 2 mM MgCl₂, 10 μ l of template and 1U of Taq DNA polymerase. The PCR amplifications were carried out with an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 49°C for 30 seconds, extension at 72°C for 80 seconds, and a final extension at 72°C for 5 minutes. When using Serogroup B specific primers the annealing and extension temperatures modified to 55°C for 30 seconds and 72°C for 30 seconds, respectively.

2.7. Electrophoresis

Amplified products were separated by agarose gel electrophoresis (1.5% agarose) at 5 v/cm for 2 h. DNA fragments were observed by UV trans-illuminator and photographed. The sizes of the amplified fragments were determined by comparing with standard DNA marker. The PCR products of selective isolates were purified and sequenced.

The concentration of DNA from *P.multocida* isolates was measured by spectrophotometer at 260 nm. The DNA was diluted 1:2, 1:10, 1:40, 1:80 and 1:160 in TE buffer, before use for PCR sensitivity determination.

3. Results

3.1. Bacteriology

All the isolates were oxidase and catalase positive and showed typical morphological and cultural properties of *P.multocida*. The *P.multocida* colonies on the blood agar were small, glistening, mucoid, dewdrop like and non-haemolytic and showed gram-negative coccobacillary organisms by gram staining. No colonies were observed when grown on MacConkey's agar.

3.2. Biochemical tests:

All the isolates fermented glucose, sucrose, sorbitol, manitol, fructose, arabinose, and maltose acid production only. A negative reaction was observed for dulcitol, lactose and salicin. All the isolates were found positive for indol and negative for urease production. Morphology of colonies and biochemical characteristics revealed that all isolates as *P.multocida*.

3.3. PCR assays

Initially PCR investigation was tried with *P.multocida* species specific primers (PM-PCR) along with capsular serogroup specific primers (CAP-PCR). All the isolates tested by PM-PCR were found to give an amplified product of 460 bp size which is species specific of *P.multocida* with the KMT1T7 and KMT1SP6 primers.

CAP-PCR was successfully optimized and applied to confirm the serogroups of *P.multocida*. The reference strains serogroups A and B yielded the expected results. The size of amplicons were 1044 bp and 760 bp respectively (Figure 1). In the present study capsular PCR typing of 53 isolates

gave an amplification product of 1044 bp and were typed as serogroup A.

The sensitivity of detection was as low as 10 ng as determined by serial dilution of template DNA. Template of unrelated organisms other than *P.multocida* produced no detectable PCR products. Negative controls were always negative. The sequence alignment of the PM-PCR and CAP-PCR fragments of the selected isolates were compared with the previous sequences in the Gene Bank, showed more than 98% similarity.

3.4. Mice bioassay

All the isolates killed mice within 24-36 h post inoculation. Giemsa stained smears prepared from heart blood samples of dead mice revealed bipolar organisms. Colonies were grown on sheep blood agar from heart blood cultures represented *P.multocida*.

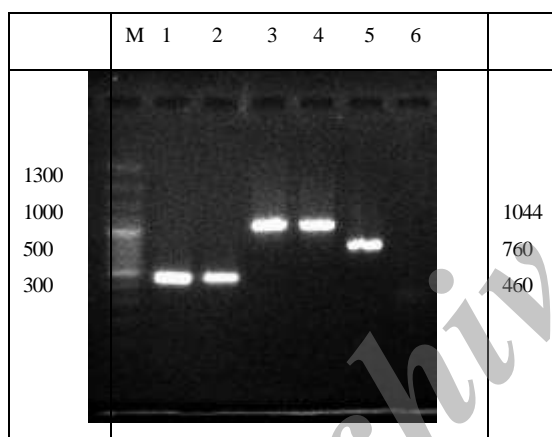


Figure 1: PCR analysis of *P. multocida* for capsular typing. Lane M: 100 bp DNA ladder (size marker); Lane1: *P.multocida* specific fragment (460 bp) – strain PMI30 (type A); Lane 2: *P.multocida* specific fragment (460 bp) – strain PMI25 (type B); Lane 3: *P. multocida* CapA fragment (1044 bp) – strain PMI30 (type A); Lane 4: CapA fragment – field isolate PM532; Lane 5: CapB fragment (760 bp)- strain PMI25 (type B); Lane 6: Negative control.

4. Discussion

P.multocida cause acute septicaemic disease characterized by high morbidity and mortality in cattle, sheep, goat and poultry, resulting in economic losses. A capsulated strain of *P.multocida* can be separated into one of the five serological groups including A, B, D, E and F based on the differences in the capsular polysaccharides. There are some differences in geographical prevalence of

the serotypes which may be of significance in the formulation of vaccines. This view was supported by Rodger (1982) who reported that immunity in the case of pasteurellosis in sheep is serotype specific and it is necessary to monitor continuously the prevalence of various serotypes so that the appropriate serotype could be incorporated in vaccines. So it becomes essential to know the serotypes of the isolates in a specific geographical region.

The system most commonly used for specific capsule antigen typing is based upon passive haemagglutination of erythrocytes sensitized by capsule antigen (Carter, 1955) which is more laborious and time consuming. In recent years, genotypic methods for bacterial identification have proved beneficial in overcoming some limitations of traditional phenotypic procedures. With the identification of the genes involved in the biosynthesis of the *P.multocida* polysaccharide capsules, serogroup-specific sequences were identified for use as primers to identify capsular groups of *P.multocida* by Townsend *et al.*, (2001). Polymerase chain reaction (PCR) has been particularly useful, which facilitates bacterial identification at any level of specificity, strain, species, genus or type-specific. The genetic differences between the capsular biosynthetic regions allow the DNA- based typing system for *P.multocida* (Kalorey *et al.*, 2008).

The present study was conducted to identification of capsular types and to know the prevalent capsular types among ovine *P.multocida* isolates from sheep in Iran. In the current study, capsular serogrouping of 52 *P.multocida* strains of sheep origin indicated that all of the ovine strains were identified as serogroup-A.

Capsular type A *P.multocida* has been isolated from sheep pneumonia cases from other countries. This serogroup has been reported from Malaysia, (Chandrasekaran *et al.*, 1991; zamri *et al.*, 1996 and Arumugam *et al.*, 2011), India (Vidhaya *et al.*, 2007) and Mexico (Vergas *et al.*, 2012). Also *P.multocida* capsular group A was shown as the dominant serogroup among avian isolates in Iran (Jabbari *et al.*, 2006) and India (Schivachandra *et al.*, 2006).

Direct PCR testing of bacterial colonies, bacterial culture lysate and mixed culture lysates as templates described in the present study

significantly reduced the time required to establish diagnosis. Moreover, the resulting amplification was as good as from purified genomic DNA.

In another study Gautam *et al.* (2004) developed a serogroup-specific PCR assay targeting the *hyaC-hyaD* genes for identification of *P.multocida* belonging to serogroup A. This PCR amplified a 564 bp product from genomic DNA prepared from cells or directly from bacterial colonies.

It was demonstrated that PCR assays can be used to identify *P.multocida* isolates from different hosts, irrespective to geographic location (Townsend *et al.*, 2001). The rapid detection and capsular serogrouping described and the versatility of PCR assay can be useful for the routine diagnosis of fowl cholera, hemorrhagic septicemia, pneumonia and other infections caused by *P.multocida*. PCR provides a sensitive, specific and rapid method for the identification of the purity of the samples from ovine respiratory system (Kalorey *et al.*, 2008; Vidhaya *et al.*, 2007).

Considering heavy losses due to pneumonic pasteurellosis in sheep farms in endemic areas of Iran, and finding that capsular type A is dominant, investigation on preparation and evaluation of an effective sheep pasteurellosis vaccine by using ovine isolates of *P.multocida* capsular type A is recommended.

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References

- Arumugam, N.D., Ajam, N., Blackall, P.J., Asiah, N.M., Ramlan, M., Yuslan, S., and Thong, K.L. 2011. Capsular serotyping of *Pasteurella multocida* from various animal hosts: a comparison of phenotypic and genotypic methods. *Trop Biomed.* 28(1): 55-63.
- Boyce, J.D., and Adler, B. 2000. The capsule is a virulence determinant in the pathogenesis of *Pasteurella multocida* M1404 (B2). *Infect Immun.* 63(6): 3463-8.
- Boyce, J.D., Chung, J.Y., and Adler, B. 2000. *Pasteurella multocida* capsule: composition, function and genetics. *J Biotechnol.* 83(1-2): 153-60.
- Brogden, K.A., Lehmkuhl, H.D., Cutlip, R.C. 1998. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. *Vet Res.* 29(3-4): 233-254.
- Carter, G.R. 1955. Studies on *Pasteurella multocida*: a haemagglutination test for the identification of serological types. *Am J Vet Res.* 16(60): 481-4.
- Carter, G.R., and Chengappa, M.M. 1981. Identification of type B and E *Pasteurella multocida* by counter immunoelectrophoresis. *Vet Rec.* 108(7): 145-6.
- Carter, G.R., and Chengappa, M.M., 1991. Rapid presumptive identification of type B *Pasteurella multocida* from haemorrhagic septicaemia. *Vet Rec.* 128(22): 526.
- Chandrasekaran, S., Hizat, K., Saad, Z., Yeap, P.C. 1991. Evaluation of combined *Pasteurella* vaccines in control of sheep pneumonia. *Br Vet J.* 147(5): 437-43.
- Chang, J.Y., Zhang, Y., Adler, B. 1998. The capsule biosynthetic locus of *Pasteurella multocida* A1. *FEMS Microbiol Lett.* 166(2): 289-96.
- Confer, A.W. 1993. Immunogens of *Pasteurella multocida*. *Vet Microbiol.* 37(3): 353-68.
- Davies, R.L., Mac Corquodale, R.M., Caffrey, B., 2003. Diversity of avian *Pasteurella multocida* starins based on capsular PCR typing and variation of the OMP A and OMP H outer membrane proteins. *Vet Microbiol.* 91(2-3): 169-82.
- De Alwis, M.C., 1992. Haemorrhagic septicaemia- a general review. *Br. Vet. J.* 148(2): 99-112.
- Gautam, J., Kumar, A.A., Singh, V.P., Vijendra, S.P., Dutta, T.K., Shivachandra, S.B., 2004. Specific identification of *Pasteurella multocida* serogroup A isolates by PCR assay. *Res Vet Sci.* 76(3): 179-85.
- Heddleston, k.l., Gallagher, J.E., Rebers, P.A., 1972. Fowl cholera: gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. *Avian Dis.* 16(4): 925-36.
- Jabbari, A.R., Saharee, A., and Esmaily, F., 2002. Characterization of avian *Pasteurella multocida* isolates by protein profiles and restriction enzyme analysis of chromosomal DNA. *Arch Razi Inst.* 54(2): 1-15.
- Jabbari, A.R., Esmaelzadeh, M., and Moazeni Jula, Gh. R., 2006. Polymerase chain reaction typing of *Pasteurella multocida* capsules isolated in Iran. *Iran J Vet Res.* 7(3): 50-55.
- Kalorey, D.R., Yuvaraj, S., Vanjari, S.S., Gunjal, P.S., Dhanawade, N.B., Barbuddhe, S.B., Bhandarkar, A.G., 2008. PCR analysis of *Pasteurella multocida* isolates from outbreak of

- pasteurellosis in Indian pigs. *Comp Immunol, Microbiol Infect Dis.* 31(6): 459-65.
- Mohammed, R.A., Abdelsalam, B.E., 2008. A Review on pneumonic pasteurellosis (respiratory maaheimiosis) with emphasis on pathogenesis, virulence mechanisms and predisposing factors. *Bulgar JVet Med.* 11(3): 139-160.
- Quinn, P.J., Carter, M.E., Marker, B., and Carter, G.R., 1994. *Pasteurella*. In: *Clinical veterinary Microbiology*. Wolfe publication. Mosby Year Book Europe Ltd, Spain. Pp. 254-258.
- Rhoades, K.R., and Rimler, R.B., 1987. Capsular serogroups of *Pasteurella multocida* isolated from avian hosts. *Avian Dis.* 31(4): 895-8.
- Rimler, R.B., and Rhoades, K.R., 1987. Serogroup F: a new capsule serogroup of *Pasteurella multocida*. *J Clin Microbiol.* 25(4): 615-8.
- Rimler R.B., 1994. Partial purification of cross-protection factor(s) from *Pasteurella multocida* isolated from turkeys. *Avian Dis.* 38(4): 778-789.
- Rimler, R.B. and Nordholm, G.E., 1998. DNA fingerprinting of *Riemerella anatipestifer*. *Avian Dis.* 42(1): 101- 5.
- Rodger, J.L., 1982. Ovine pasteurellosis vaccines. *Vet Res.* 111(12): 283.
- Sambrook, J., Fritsch, E.F., and Maniatis, T., 1989. *Molecular cloning- A laboratory manual*. Second Edition. Cold Spring Harbor Laboratory Press. Pp: 1641-1708
- Shivachandra, S.B., Kumar, A.A., Gautam, R., Vijendra, P., Singh, M., Saxena, K., and Srivastava, S.K., 2006. Identification of avian strains of *Pasteurella multocida* in India by conventional and PCR assays. *Vet J.* 172(3): 561-564.
- Townsend, K.M., Dowkins, and Papadimitriou, J.M., 1997. REP-PCR analysis of *Pasteurella multocida* isolates that cause haemorrhagic septicaemia. *Res Vet Sci.* 63(2):151- 5.
- Townsend, K.M., Boyce, J.D., Chung, J.Y., Frost, A.J., and Adler, B., 2001. Genetic organization of *Pasteurella multocida* cap loci and development of a multiplex capsular PCR typing system. *J Clin Microbil.* 39(3): 924-9.
- Vergas, E.S., Sanches, V.V., Espinosa, J.L.Z., Dibarrat, J.A., Romero, F.A., and Abascal, E.N., 2012. Identification of *Pasteurella multocida* capsular types isolated from rabbits and other domestic animals in Mexico with respiratory diseases. *Trop Anim Health Prod.* 44(5): 935-7.
- Vidhya, M., Daniel, N., Manohar, W., and Dhinakar, G., 2010. Molecular identification of *Pasteurella multocida* isolated from sheep by capsular PCR typing. *Tamilandu J Vet Anim Sci.* 6: 81-87.
- Wilson, M.A., Morgan, M.J., and Barger, G.E., 1993. Comparison of DNA fingerprinting and serotyping for identification of avian *Pasteurella multocida* isolates. *J. Clin. Microbiol.* 31(2): 255-259.
- Wilson, M.A., Duncan, R.M., Nordholm, G.E., Berlowski, B.M., 1995. *Pasteurella multocida* isolated from wild birds of north America: A serotype and DNA fingerprint study of isolates from 1978 to 1993. *Avian Dis.* 39(3): 587-93.
- Zamri, S., Effendy, A.W.M., Maswati, M.A., 1996. The goat as a model for studies of pneumonic pasteurellosis caused by *Pasteurella multocida*. *Br Vet J.* 152(4): 453-8.