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ORIGINAL ARTICLE

Molecular characterization of the capsular antigens of *Pasteurella multocida* isolates using multiplex PCR

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Abstract 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. During the period from 15th February, 2014 to 15th April, 2015, 425 nasopharyngeal swabs and 175 lung and spleen samples were collected and examined by conventional methods, 80 strains (18.82%) of *P. multocida* were isolated from the calves, sheep and goat with respiratory manifestation. Meanwhile, 77 strains (44%) were isolated from emergency slaughtered animals. All the recovered strains were positive for specific PCR for detection of *P. multocida* strains previously identified as *P. multocida* by standard microbiological techniques. Multiplex PCR for molecular typing of the capsular antigens of the recovered *P. multocida* revealed positive amplification of 1044 bp fragments specific to the capsular antigen type A with 105 strains (66.88%), and amplification 511 bp fragments of the capsular antigen type E with 52 strain (33.12%) and absence of B, D and F antigens.

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Multiplex PCR for molecular typing of the capsular antigens of *P. multocida* can be used as a simple, sensitive, rapid, reliable technique instead of the serological techniques for identification of the capsular antigens of *P. multocida*

1. Introduction

Pasteurella multocida is a Gram-negative bacterium responsible for Pasteurellosis (acute septicaemic disease characterized by high morbidity and mortality rate as well as severe economical losses) in cattle, sheep, goat and poultry (Prabhakar et al., 2012; Jakeen et al., 2016). It has a zoonotic impact in human; it can cause soft tissue infection after animal bites or via inhalation causing respiratory tract infection.

Based on its capsular antigens *P. multocida* had been classified into five groups (capA, B, D, E and F) (Espinosa et al., 2012; Prabhakar et al., 2012). *P. multocida* is incriminated in many other disease conditions such as mastitis, meningitis, peritonitis, atrophic rhinitis and ear infections due to the effect of *P. multocida* toxins (Atashpaz et al., 2009; Prabhakar et al., 2010).

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. multocida* and the other *Pasteurella* species specially the *Pasteurella haemolytica* (*Mannheimia haemolytica*) (Berge et al., 2006; Bell, 2008; Rajeev-Gautam et al., 2006).

Therefore, the use of molecular techniques, specially the polymerase chain reaction (PCR) for molecular detection and characterization of the capsular antigens of the *P. multocida* is very important for rapid and specific detection and characterization of the organism which play an important role in the control of the disease among the farm animals and reducing the economical losses (Rajeev-Gautam et al., 2006; Jakeen et al., 2016). Therefore, the current study is aimed to detect and characterize the recovered strains of *P. multocida* from samples collected from different abattoirs located in Riyadh, Kingdom of Saudi Arabia.

2. Materials and methods

2.1. Samples

During the period from 15th February, 2014 to 15th April, 2015, 425 nasopharyngeal swabs were collected under aseptic conditions from calves (n = 200) from 6 to 12 months age, sheep (n = 125) 6 to 12 months and goat (n = 100) from 1 to 2 years age suffering from respiratory manifestations. Also 175 lung samples were collected from emergency slaughtered calves (n = 75), sheep (n = 50) and goat (n = 50). The samples collected from different abattoirs located in Riyadh, Kingdom of Saudi Arabia and transferred under complete aseptic condition to the College of science laboratory at King Saud University for standard bacteriological techniques and for molecular detection and characterization of *P. multocida*.

2.2. Standard bacteriological examination

All the collected samples were cultured primary into casein sucrose yeast broth (Oxoid) for 6–8 h and a loopful from each broth were sub-cultured on to blood agar and MacConkey agar media and incubated for 48 h at 37 °C. The recovered strains of *P. multocida* were completely identified with API 20 E tests (BioMérieu) according to Jakeen et al. (2016).

2.3. Pathogenicity test

For evaluation of the pathogenicity of the recovered *P. multocida* isolates each strain was cultured in brain heart infusion broth and after 24 h incubation, the turbidity of each tube was adjusted to McFarland's tube no 4. From each test tube 0.1 ml of 24 h broth culture were inoculated into 3 mice intramuscularly and the mice were kept under observation for 72 h. Dead mice were subjected to postmortem examination and blood films were prepared and stained with Leishman's stain and examined for the presence of the characteristic bipolarity of *P. multocida*.

2.4. Serotyping of the somatic and capsular antigens using ELISA

ELISA had been carried out according to Jakeen et al. (2016) using somatic antigens and capsular antigens as coating antigens and standard positive control hyperimmune serum prepared in rabbit according to the previously reported techniques by OIE (2008).

2.5. Molecular detection of P. multocida by PCR

Molecular detection of *P. multocida* was carried out according to the previously reported method by Jakeen et al. (2016) using Qiagen kits, species primers for detection of *P. multocida*; forward primer KMT1T7 (5′-ATC CGC TAT TTA CCC AGT GG-3′), reverse primer KMT1SP6 (5′-GCT GTA AAC GAA CTC GCC AC-3′). The molecular characterization of the capsular antigens of *P. multocida* was carried out by multiplex PCR using specific primer pairs for different serogroups (A, B, D, E and F), previously reported by Townsend et al. (1998).

3. Results

The collected samples from animals with respiratory manifestation revealed positive isolation of 80 strains (18.82%) of *P. multocida*, while, 77 strains (44%) were isolated from lung

Table 1 Rate of isolation of *P. multocida* from different species of animals suffering from respiratory manifestation.

Types of samples	Samples from animals with respiratory manifestation	Number of samples	Percent of isolation of *P. multocida*	
			Number	Percentage (%)
Sterile nasopharyngeal swabs	Calves	200	35	17.5
	Sheep	125	26	20.8
	Goat	100	19	19
	Total number	425	80	18.82
Lung and spleen	Calves	75	30	40
	Sheep	50	22	44
	Goat	50	25	50
	Total number	175	77	44

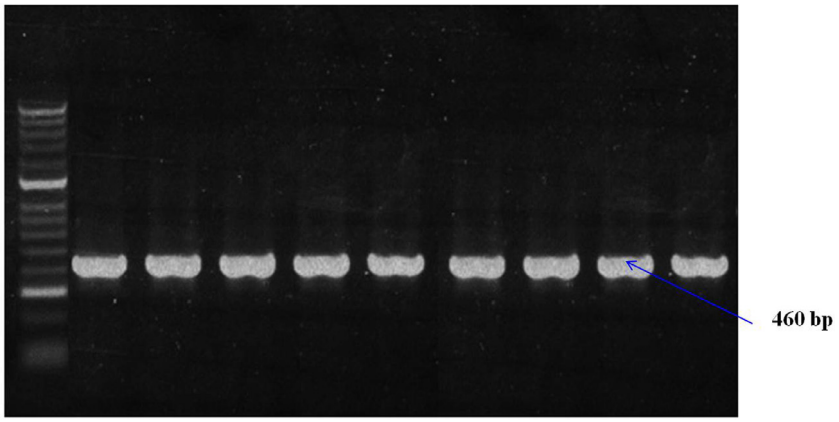

Figure 1 Agarose gel electrophoresis showing amplification of 460 bp fragments specific for *P. multocida* field isolates.

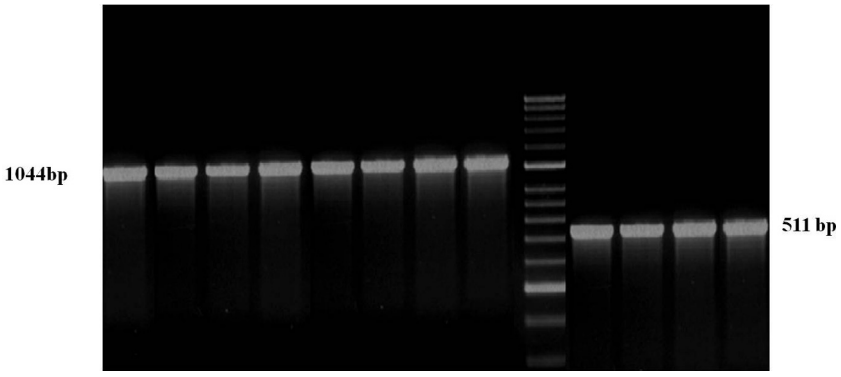

Figure 2 Agarose gel electrophoresis showing amplification of 1044 bp fragments specific to the capsular antigen type A and 511 bp fragments specific to the capsular antigen type E.

and spleen of emergency slaughtered animals as shown in Table 1. The characteristic colonial morphology of *P. multocida* had been observed with all the recovered strains. Moreover, all the recovered strains showed severe pathogenicity and killed all the inoculated mice within 24–72 h and the characteristic bipolarity could be observed in the stained blood and spleen smear prepared from the dead mice with Leishman's stain. The results of ELISA test using somatic and capsular antigens as coating antigens to test the serum samples collected from all animals with respiratory manifestation and emergency slaughtered animals were 64% and 68% respectively.

Molecular identification of the recovered strains with the specific primers KMT1T7 and KMT1SP6 revealed positive amplification of 460 bp fragments with all the recovered strains previously identified as *P. multocida* by standard microbiological techniques as shown in Fig. 1. Multiplex PCR for molecular typing of the capsular antigens of the recovered *P. multocida* revealed positive amplification of 1044 bp fragments specific to the capsular antigen type A with 105 strains (66.88%), and amplification 511 bp fragments of the capsular antigen type E with 52 strain (33.12%) as shown in Fig. 2 and absence of B, D and F antigens.

4. Discussion

The infections with *P. multocida* most commonly occur as secondary infections to the viral infection and other diseases conditions (Worarach et al., 2014). The interaction between bacterial infection and viral affection in cases of pneumonia of sheep and goats is usually occurring. A primary viral pneumonia may be an insignificant disease until the intervention of a secondary pasteurellosis which converts it into an outbreak of pneumonia of major economic importance (Rad et al., 2009).

The present study is aimed to detect and characterize the recovered strains of *P. multocida* from samples collected from different abattoirs located in Riyadh, Kingdom of Saudi Arabia on molecular basis. 157 strains of *P. multocida* were isolated from the examined samples all of them showed positive results with, catalase, oxidase, ornithine decarboxylase, indole and negative for urea hydrolyze, gelatin liquefaction, and cannot ferment lactose and mannitol sugar (Jakeen et al., 2016). To overcome the disadvantage of the conventional methods used for diagnosis of *P. multocida* PCR assay reported by Townsend et al. (1998) using KMT1T7 and KMT1SP6 primers has been used to rapidly confirm the results of the standard bacteriological methods. All the recovered strains showed positive amplification of 460 bp fragments specific for *P. multocida* within few hours which indicate the higher sensitivity and specificity of the molecular techniques (Kumar et al., 2009; Jakeen et al., 2016). Multiplex PCR has been carried out in the current study for molecular characterization of the capsular antigens to overcome the disadvantage of the serological methods used for the typing of *P. multocida*. Positive amplification of 1044 bp fragments specific to the capsular antigen type A with 105 strains (66.88%), and amplification 511 bp fragments of the capsular antigen type E with 52.

5. Conclusion

Molecular techniques based on multiplex PCR for molecular typing of the capsular antigens of *P. multocida* can be used as a simple, sensitive, rapid, reliable technique instead of the serological techniques used for identification of the capsular antigens of *P. multocida*.

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References

Atashpaz, S., Shayegh, J., Hejazi, M.S., 2009. Rapid virulence typing of *P. multocida* by multiplex PCR. Res. Vet. Sci. 87 (3), 355–357.

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.

Bell, S., 2008. Respiratory disease in sheep. 2. Treatment and control. Br. Vet. Assoc. 30 (5), 278–283, In-Practice.

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

Espinosa, I., Baez, M., Vichi, J., Martinez, S., 2012. Antimicrobial resistance and genes associated to the host-microbe interaction of *P. multocida* isolates from swine in Western Cuba. Rev. Salud Anim. 34 (3), 151–158.

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.

Kumar, P., Singh, V.P., Agrawal, R.K., Singh, S., 2009. Identification of *P. multocida* isolates of ruminant origin using polymerase chain reaction and their antibiogram study. Trop. Anim. Health Prod. 41 (4), 573–578, 24.

OIE, 2008. Haemorrhagic septicaemia Chapter 2. 4. 12. Terrestrial Manual. Page 739–751. Office International Des Epizooties (OIE), Parise, France.

Prabhakar, P., Thangavelu, A., Kirubaharan, J.J., Chandran, N.D.J., 2010. Isolation and characterization of *P. multocida* isolates from small ruminants and avian origin. Tamilnadu J. Vet. Anim. Sci. 8 (3), 131–137.

Prabhakar, P., Thangavelu, A., Prabhakar, T.G., Kirubaharan, J.J., Chandran, N.D.J., 2012. Rapid virulence typing of *P. multocida* in sheep isolates of Tamil Nadu. Indian J. Anim. Sci. 82 (4), 351–354.

Rad, M., Movassaghi, A.R., Sharifi, K., Naseri, Z., Seifi, H.A., 2009. Two outbreaks of *P. multocida* septicemia in neonatal lambs. Comp. Clin. Pathol. 20 (1), 57–59.

Rajeev-Gautam, Dutta, T.K., Kumar, A.A., Shivachandra, S.B., 2006. Molecular typing of Indian isolates of *P. multocida* serogroup-A from different animal species. Indian J. Anim. Sci. 76 (11), 867–872.

Townsend, K.M., Frost, A.J., Lee, C., Papadimitriou, J.M., Dawkins, H.J.S., 1998. Development of PCR assays for species and type-specific identification of *Pasteurella multocida* isolates. J. Clin. Microbiol., 1096–1100

Worarach, A., Promto, G., Pathanasophon, P., 2014. Molecular characterization of *Pasteurella multocida* isolates that cause haemorrhagic septicaemia in Thailand using pulsed-field gel electrophoresis. Thai J. Vet. Med. 44 (1), 23–29.