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Article

Detection of *Pasteurella multocida*, *Mannhemia haemolytica*, *Histophilus somni* and *Mycoplasma bovis* in cattle lung

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Abstract:

In this study, it was aimed to determine of *P. multocida, M. haemolytica, H. somni* and *M. bovis* in macroscopically healthy cattle lungs by PCR. The study was carried out on 82 macroscopically healthy cattle lung. DNA extraction was performed to the lung samples. PCR was then performed using all specific primers. By molecular evaluation, positive results were achieved for *P. multocida*, *M. haemolytica*, *H. somni* and *M. bovis* in 4 (4.8 %), 4 (4.8 %), 6 (7.3 %) and 3 (3.6 %) of the samples, respectively. Mix infections were detected in five samples. Of the samples, two were positive for both *P. multocida* and *M. haemolytica,* two were positive for both *M. haemolytica* and *H. somni* and one was positive for both *P. multocida* and *H. somni.* However, a positive sample, which carried all of pathogens, was not detected. In conclusion, *P. multocida, M. haemolytica, H. somni* and *M. bovis* are the important opportunistic pathogens of respiratory tract in cattle and these pathogens have a major role during infections. But multifactorial nature of bovine respiratory disease and immune system affected the formation of the disease. Hence, firstly cattle's immunity should be strengthened and other conditions should be kept under control.

Key words: Cattle, Molecular analysis, Lung, Respiratory disease.

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Introduction

Bovine respiratory disease (BRD) is one of the major health problems in calves and adult cattle, and has great economic impact on the cattle industry[1-3]. BRD in herds causes economic losses due to increased treatment costs, decreased production and culling[4]. BRD has a complex etiology, which involves bacterial and viral agents. Additionally, some predisposing factors such as management failures, environmental and host defense problems are influential on infection occurrence[5].

The most frequent bacterial agents isolated from respiratory disease are *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*), *Histophilus somni* (*H. somni*) and *Mycoplasma bovis* (*M. bovis*)[6,7]. *Pasteurella multocida* is one of the primary bacterial pathogens and leads to clinical symptoms during BRD in neonatal calves and cattle. The bacterium, which is detected not only in infected but also healthy cattle, is isolated from lung, nasopharyngeal and nasal swabs and trans-tracheal washes. Therefore, diagnose of *P. multocida* becomes an issue, if clinical symptoms associated with pneumonia are detected in cattle[8].

Similarly, *M. haemolytica* is normally presented in nasal pharyngeal mucosa in healthy cattle. However, the bacterium becomes a pathogen under inadequate conditions such as nutritional deficiency and overcrowded housing, and viral infections. Following to rapid proliferation of *M. haemolytica* within the infected lung, severe fibrinopurulent bronchopneumonia is presented. Additionally, it produces a potent leukotoxin that destroys the macrophages and neutrophils[9,10]. Because of these properties, this bacterium is accepted as the most harmful pathogen for lung.

H. somni is a bacterium which is normally presented not only in respiratory but also in reproductive tract. Similar with mentioned pathogens above, *H. somni* is also causing infections such as thrombotic meningoencephalitis (TME), pneumonia, septicemia, mastitis, arthritis, myocarditis and reproductive infections under inappropriate conditions with clinical symptoms[5,11-13].

M. bovis is not only respiratory disease but also arthritis, mastitis, genital infections and abortus[3]. Moderate infections in cattle has the potential to cause an infection with severe

clinical manifestations, as well as difficulty diagnosis; penicillin and its derivates are also an important problem in cattle breeding enterprises with the resistance mechanism of antibiotics[14]. At the same time, the rapid spread of bacteria in the cattle herd was a result of *M. bovis* makes it important[15].

BRD is known as polymicrobial infection in cattle herds and mainly recorded in younger cows[13]. Thus, diagnosis of BRD is required to use different methods (conventional and molecular) to determine the bacteria that are effective in etiology. In particular, the use of different media, incubation conditions (temperature and O_2 ratio), and differences between methods in conventional diagnostic methods require the use of faster methods. Molecular diagnosis of the pathogens based on Polymerase Chain Reaction (PCR) techniques can be used for identification and detailed evaluations. Molecular techniques, which are more sensitive than bacteriological methods, are preferred especially for direct identification of pathogens from tissue samples [8,9,15,16].

The aim of this study was to determine of *P. multocida, M. haemolytica, H. somni* and *M. bovis* of macroscopically healthy cattle lungs by PCR.

Material and methods

Sampling procedure

A total of 83 lung samples were collected from the different slaughterhouses. A piece of lung sample was taken from lungs without any lesions and placed in sterile tubes transported to the laboratory in cool chain.

Culture and DNA extraction

A piece of sample was taken and placed in eppendorf tube. Briefly, each sample was put into sterile petri dishes and then samples were break into parts using sterile bistouries and pens. Broth culture was only used for *M. bovis*. A piece of lung sample for *M. bovis* isolation inoculated in PPLO broth medium and incubated at 37 ^{o}C in %5 CO_2 for 5 d. PPLO broth cultures were used for *M. bovis* DNA extraction, lyzed lung samples were used for other bacteria DNA extraction. DNA was extracted from the lung samples by using genomic DNA purification kit (Qiagen-DNeasy Blood and Tissue Kit-USA). Manufacturer instructions

were followed.

Polymerase Chain Reaction

PCR procedures, which involved cycle conditions and reaction mixture, were performed according to previous reports[17,18,19] (Table 1). The reaction mixture was prepared with a total volume of 50 µl contained 3 mM $MgCl_2$, 200 µl dNTP, 0.5 µM each of primer and 1.25 units Taq DNA polymerase (Vivantis, MY) with minor revisions for pathogens. Extracted DNA (1 µL) was used as template. Amplification was carried by thermalcycler (The SuperCycler Trinity, Kyratech, AU). All samples of PCR amplification products (10 µL) were subjected to electrophoresis. DNA was visualized by UV fluorescence after staining with ethidium bromide.

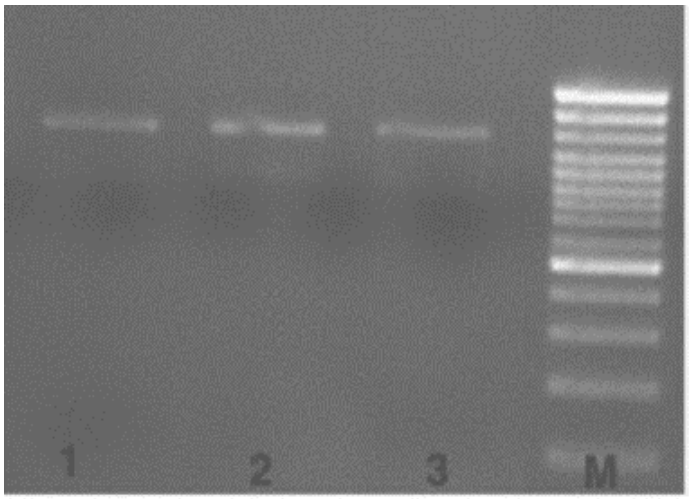
Table 1: PCR conditions and oligonucleotids sequences

Pathogen	Cycle condition (°C/min)	Oligonucleotids	Base pair (bp)	Reference
P. multocida	94/1 69/1 30 cyc 72/1	F:GGCTGGGAAGCCAAATCAAAG R:CGAGGGACTACAATTACTGTAA	1432	Miflin and Blackall, 2001
M. haemolytica	94/1 55/1 30 cyc 72/1	F:TGTGGATGCGTTTGAAGAAGG R:ACTTGCTTTGAGGTGATCCG	1145	Akan *et al,* 2006
H. somni	94/1 55/1 35 cyc 72/1	F:GAAGGCGATTAGTTTAAGAG R:TTCGGGCACCAAGTRTTCA	400	Angen *et al,* 1998
M. bovis	94/1 54/1 30 cyc 72/1	F: TATTGGATCAACTGCTGGAT R: AGATGCTCCACTTATCTTAG	447	Foddai *et al,* 2005

Results

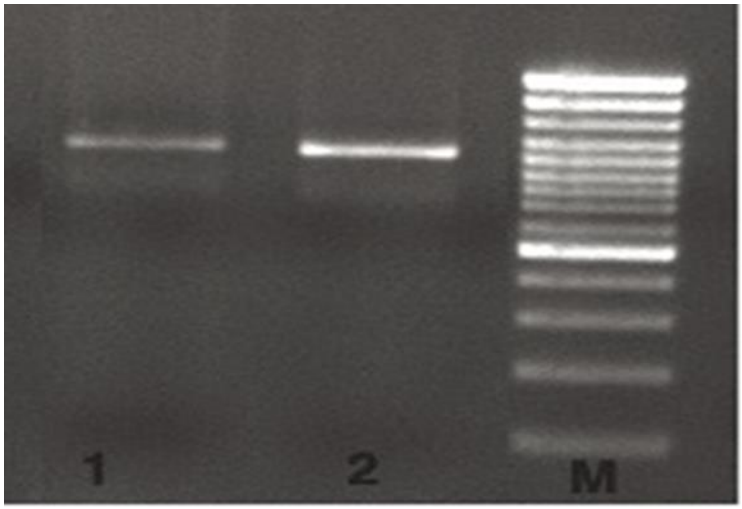
In molecular evaluation, positive results were achieved for *P. multocida*, *M. haemolytica*, *H. somni* and *M. bovis* in 4 (4.8 %), 4 (4.8 %), 6 (7.3 %) and 3 (3.6 %) of the samples, respectively (Figure 1-3). Mix infections were detected in five samples. Of the samples, two were positive for both *P. multocida* and *M. haemolytica*, two were positive for both *M. haemolytica* and *H. somni* and one was positive for both *P. multocida* and *H. somni.* However, a positive sample, which carried all of the pathogens, was not detected.

Figure 1: Molecular evaluation of *P.multocida*



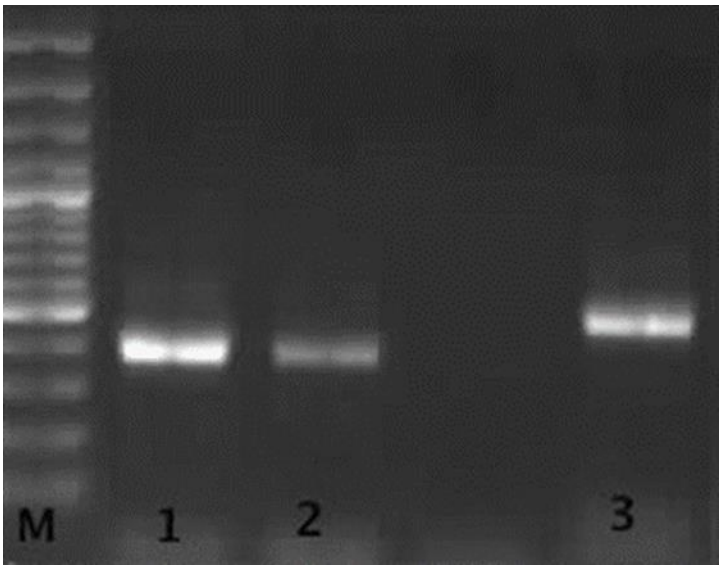
M= Marker (100 bp DNA Ladder Plus), 1-3= *P.multocida*

Figure 2: Molecular evaluation of *M. haemolytica*



M= Marker (100 bp DNA Ladder Plus), 1-2= *M. haemolytica*

Figure 3: Molecular evaluation of *H. somni* and *M.bovis*



M= Marker (100 bp DNA Ladder Plus), 1-2= *H. somni*, 3= *M.bovis*

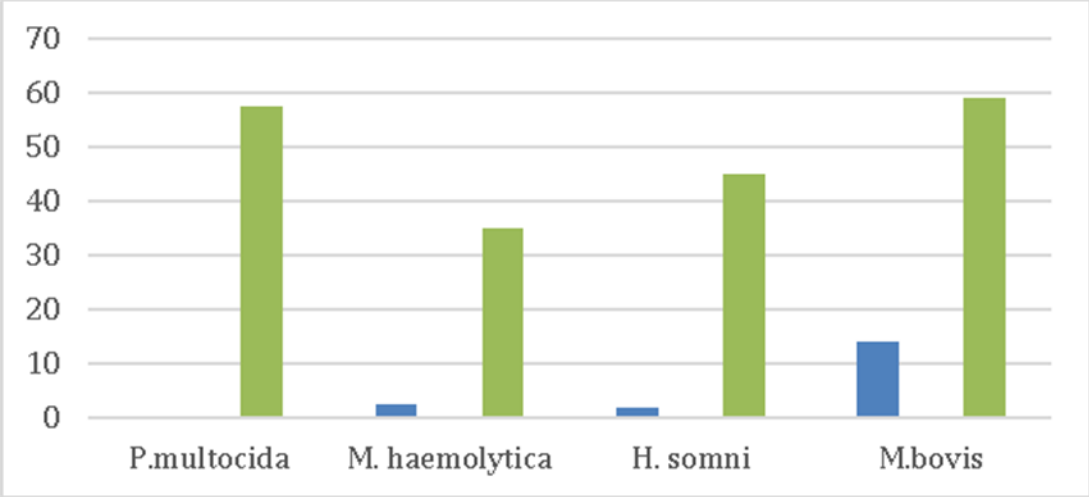
Discussion

Bovine respiratory diseases, which cause economic losses due to production decrease and culling, have major importance in cattle herds. Although, cattle of all ages and sex are susceptible to the disease, it is more harmful for calves[6,20,21]. Bacteria that cause respiratory infections can be transmitted to sensitive animals from healed or immunologically strong animals (no clinical signs)[2,22]. In addition to pathogens, some predisposing factors such as overcrowded and bad-ventilated barns, inadequate feeding and other infectious diseases increased the infection risk. In these herds, transmission usually occurs horizontally[7,9,23]. Previous studies were usually focused on the detection of *P. multocida*, *M. haemolytica*, *H. somni* and *M. bovis* in the tonsils, nasopharynx and upper respiratory tract in carrier animals[2,24,25]. Various results about the presence of pathogens in cows were achieved in the reports. Positivity of *P. multocida*, *M. haemolytica, H. somni and M. bovis* in cattle varied between 0.4-57.4 %, 1.6-35 %, 2-45 % and 14-59 % in previous reports (Figure 4)[9,19,26-30].

In the present study, the number of positive animals identified for *P. multocida* was found lower than Onat *et al*[28] but was found higher than others[24,27]. Positivity of *M. haemolytica* was found lower than some other works[2,27,29], but was found higher than Hajikolaei *et al*[20]. In terms of positivity *H. somni* and *M. bovis* was found different from other studies. In studies, in both bacteria was evaluated different diagnostic method in pneumonic

cows[15,30,31]. Additionally, the variation among the results may be associated with difference in diagnostic methods, vaccination, and bacterial properties[2,23,29]. For instance, conventional culture methods can be inadequate in detection of the pathogens in healthy cows due to lower bacterial count in the samples. Likewise, vaccination can reduce bacteria carriage and lesions[16,32,33]. In addition, detection of some respiratory system pathogens as *H. somni* and *M. bovis* in culture media is difficult although the samples are collected from infected cows[9,19]. Thus, PCR tests, which involve specific primers for 16S rRNA, are suggested for identification of this bacterium during mix respiratory infections[5,13]. Therefore, genetic material basis molecular technics, which allow the detection of the pathogens even though lower bacterial count, would be preferable rather than culture methods in determining of carrier cows [16,34]

Figure 4: Proportional change of pathogens according to studies [9,20,24,27,29,30,31]



The presence of these bacteria in the absence of clinical symptoms in animals or macroscopic lesions in the necropsy supports the opportunistic character of these bacteria. However, in these cases, histopathological examinations should be done and the animal's health status should be questioned. Additionally, because the occurrence of the disease has association with carriage[22,24,27,35], detection of reservoir animals is important for reduction of the risk in herds. So that, detection of carrier cows, which have potential risk for contamination, is an approach for control[22].

Conclusions and implications

In conclusion, *P. multocida*, *M. haemolytica*, *H. somni* and *M. bovis*, which cause economic losses and death in animals, are also important opportunistic pathogens. Therefore, the

immune system should be developed by vaccination in animals. Moreover, housing conditions and management, awareness of the staff (owner) should be improved to establish an effective and sustainable control program for respiratory system diseases.

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