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RESEARCH ARTICLE

Pathological, cytological, microbiological and molecular investigations of pneumonia caused by *Pasteurella multocida* and *Mannheimia haemolytica*

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Sığırlarda *Pasteurella multocida* ve *Mannheimia haemolytica* kaynaklı pnömonilerin patolojik, sitolojik, mikrobiyolojik ve moleküler olarak araştırılması

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Öz

Amaç: Sığır yetiştiriciliğinde Pasteurella multocida ve Mannheimia haemolytica'nın sebep olduğu pnömoniler yol açtıkları ekonomik kayıplar nedeniyle oldukça önemlidir. Bu çalışmada mikrobiyolojik yöntemler ve real time PCR ile P. multocida ve M. haemolytica'nın neden olduğu belirlenen pnömonili sığır akciğerlerinde ve nasal swaplarda belirlenen sitolojik bulguların ortaya konulması amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmada sığır pnömonik akciğerlerinde bakteriyolojik kültür, histopatolojik, sitolojik ve real time PCR teknikleri kullanıldı. Real time PCR ile M.haemolytica ve P.multocida belirlenen pnömoni olgularında sitolojik bulgular değerlendirilerek diğer pnömoni tipleri ile karşılaştırıldı.

Bulgular: Burun swap ve akciğer örneklerinden hazırlanan smear örneklerinde sitolojik bulgular kaydedildi. Pnömoni belirtisi görülen 233 sığırın 23'ünde (%9.87) P.multocida ve M. haemolytica'ya bağlı pnömoni belirlendi. Fibrinli bronkopnömonili hayvanlar içerisinde yaklaşık % 51,11'inde P.multocida ve M.haemolytica olduğu belirlendi. Bu olguların sitolojik incelemelerinde diğer pnömonilere kıyasla nötrofil lökosit sayısının arttığı tespit edildi. Nötrofillere ilave olarak; P.multocida ve M.haemolytica belirlenemeyen diğer fibrinli bronkopnömoni olgularıyla karşılaştırıldığında bu olgularda lenfosit ve silialı epitel hücrelerin sayısının önemli derecede arttığı belirlendi.

Öneri: Çalışmanın sonuçları Pasteurella multocida ve Mannheimia haemolytica'nın sebep olduğu pnömonilerin sığır solunum sistemi enfeksiyonlarının önemli bir bölümünü oluşturduğunu ve fibrinli pnömoniye neden olduğunu göstermiştir. Elde edilen sitoloji sonuçları pnömonilerin tiplendirilmesinde klinik olarak değerlendirilebilir.

Anahtar kelimeler: Pasteurella multocida, Mannheimia haemolytica, sitoloji, mikrobiyoloji, real time pcr

Abstract

Aim: In cattle breeding, pneumonia caused by Pasteurella multocida and Mannheimia haemolytica is very important due to the economic losses caused. In this study, it was aimed to reveal cytological findings in pneumonic bovine lungs and nasal swaps due to P. multocida and M. haemolytica determined by microbiological methods and real time PCR.

Materials and Methods: In this study, bacteriological culture, histopathological, cytological and real time PCR techniques were used in pneumonic bovine lungs. Cytological findings were evaluated in cases of pneumonia whose etiological agent was identified as M.haemolytica and P.multocida by real time PCR and were compared with other pneumonia types

Results: Cytological findings were recorded in smear samples prepared from nasal swaps and lung samples. Pneumonia caused by P. multocida and M. haemolytica was detected in 23 cases (9.87%) out of 233 cattle with signs of pneumonia. P.multocida and M.haemolytica were determined to occur in about 51.11% of the animals with fibrinous pneumonia. During the cytological examination of these cases, neutrophils were seen increased in number compared to the other types of pneumonia. In addition to neutrophils, the number of lymphocytes and ciliated epithelial cells was also significantly increased in these cases compared to the other fibrinous pneumonia cases in which P.multocida ve M.haemolytica was not detected.

Conclusion: The results of this study showed that P. multocida and M. haemolytica cause a fibrinous type of pneumonia and constitute an important portion of cattle respiratory diseases. Clinically, cytology results may be evaluated for the typing of pneumonia.

Keywords: Pasteurella multocida, Mannheimia haemolytica, cytology, microbiology, real-time pcr

Introduction

It is well known that diseases of the respiratory system in livestock animals cause great economic losses all over the world (Yates 1982, Frank 1986, Ozturk et al 1996, Batmaz 2006, Erbas and Kaya 2008, Akıllı et al 2012). Among the respiratory system diseases, pasteurellosis and mannheimosis holds an important place in cattle breeding. The damaging effects of the disease, which typically result in fibrinous pneumonia with pleural adherence, become mostly worse by other factors such as bacteria and viruses (Yates 1982, Alexander et al 1989, Maity and Deb 1991, Altun 2015). Death of animals, decreased meat and milk production, and increased maintenance expenditures are the main reasons for the economic losses.

Pasteurella agents are Gram (-) bacteria and characteristically stained in bipolar shape in tissue smears. They easily grow in agar containing blood or serum in aerobic or facultative anaerobic conditions. These bacteria have endotoxin but do not cause proteolytic effects (Gunduz and Erganis 1998, Arda et al 1999, Quinn et al 2016).

Pasteurellosis is a widespread disease and can easily be contracted by respiratory and digestive systems as well as conjunctiva and skin wounds. The bacteria live as a facultative pathogen in the upper respiratory tract and pharynx of healthy animals and can cause infection when the defense of the animal is impaired (Hazıroglu et al 1997, Çiftçi et al 2015). The disease caused by the agent is called "shipping fever" as it appears after the cattle are transported through long distances especially under non-optimal conditions (Arda et al 1999, Çiftçi et al 2015). Stress factors such as constricted housing, hungriness, sudden climatic changes, management/nutrition mistakes, and viral infections such as IBR, PI-3, and respiratory syncytial virus diseases predispose the animals and facilitate the occurrence of the disease (Yates 1982, Alexander et al 1989, Maity and Deb 1991, Caswell and Williams 2007).

In pasteurella infected animals, grossly dark-red to gray viscous or hepatized areas with occasional necrotic foci are generally seen especially in the cranial lobes of lungs. Edema and dilation are seen in the interlobular septum. Infected lobulus in different time periods in pasteurellosis and mannheimosis can be found collaterally. This gives a mottled appearance to the cross-section of the infected lung (Ozturk et al 1996, Hazıroglu et al 1997). Pleura covering the surface of the lesions are also mostly infected and hence adheres to the rib cage. Although it is not a rule, it is generally accepted that M. haemolytica causes fibrinonecrotic bronchopneumonia and P. multocida causes fibrinopurulent bronchopneumonia. In the microscopic view of the infected areas, oat-cells with long and round nucleus are typically seen (Hazıroglu et al 1997, Amrine et al 2014).

Identification of the causative agents in Pasteurella suspicious cases can be made by conventional bacteriological culture. However, advances in molecular biology led to the development of fast and reliable diagnostic methods. Polymerase chain reaction (PCR) can now be used as an alternative to culture and antigen-antibody binding based tests in the diagnosis of many bacterial diseases (Kamp et al 1996, Lichtensteiger et al 1996, Kılıc and Muz 2004). On the other hand, a cytological examination may still be needed to determine the type and severity of the infection that can in some occasions help to decide what measures are needed to be taken for the treatment of the disease (Oruc and Tuzcu 2010).

In this investigation, we aimed to determine of P. multocida and M. haemolytica in cattle in the city of Sivas, Turkey. The use of real-time PCR technique in diagnosis was tested and compared to the conventional microbiological culture. Cytological and histopathological examinations were also performed to better identify the characteristics of pneumonia caused by P. multocida and M. haemolytica in cattle.

Material and Methods

In The study materials were nasal swabs and lung samples collected from 233 cattle which showed pneumonia symptoms among the total of 3270 slaughtered cattle during a 12-month period in the city of Sivas, Turkey. In cytological examinations, only tissues that were identified microbiologically by M.haemolytica and P. multocida and identified by Real Time PCR were used.

Cytological examinations

In the gross examination, 233 cattle were diagnosed with pneumonia. Touch smears from these animals and the smears prepared from nasal swabs before the slaughter were prepared. All smears were fixed with methanol and stained with Papanicolau and May-Grunwald Giemsa routinely. A total of 200 cells in each sample were counted and the number of cell types was recorded.

Histopathological investigations

Pneumonia suspicious lung samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Then, serial sections in 5µm thickness were cut and stained routinely with hematoxylin and eosin for microscopic evaluation. Gram staining was also performed.

Microbiological investigations

The lung samples were collected and brought to the micro-

Table 1. The primer and probe sequences used in the detection of P. multocida 16S r RNA gene and M. haemolytica sod A gene

P. multocida	F primer 5′-ATAACTGTGGGAAACTGCAGCTAA-3′ R primer 5′-GGTCCCACCCTTT(A/C)CTCCTC-3′ MGB probe PMA2 5′-6FAM-CCGCGTA(A/T)TCTCTMGBNFQ-3′
M. haemolytica	F primer 5′-AGCAGCGACTACTCGTGTTGGTTCAG-3′ R primer 5′-AAGACTAAAATCGGATAGCCTGAAACGCCTG-3′

biology laboratory under a cold chain for bacterial analysis. Samples were inoculated into 7% sheep blood agar and Mac Conkey agar and incubated at 37 °C. Pure cultures of suspicious colonies were identified based on the characteristics of gram staining, bipolar staining, hemolysis, catalase, oxidase, coagulase, Mac Conkey agar reproduction, indole, urease, VP, movement, H2S, and nitrate reduction (Quinn et al 2016).

Real-time PCR analysis

Total DNA isolation from tissue and swab samples was performed by the High Pure PCR Template DNA extraction kit (Roche catalog number: 11796828001). The isolated DNA samples were stored at -20 °C until PCR analysis was performed.

In real-time PCR analysis, Light Cycler Fast Start DNA Master SYBR Green I kit (Roche catalog number: 03003230001) and Light Cycler TaqMan Master (Roche catalog number: 04535286001) were used. Detection of Pasteurella multocida and Mannheimia haemolytica agents was based on the methods described by Corney et al. (2007) and Guenther et al. (2008) with some modifications. P. multocida 16S rRNA gene and M. haemolytica sod A gene were targeted for detection. The primer and probe sequences were given in Table 1. Real-time PCR technique was performed as follows for P. multocida; 2 µl Taq Man Master mix (containing Taq-polymerase), 1µl (0.2µM) probe, 2µl (50ng/µl) template DNA and 13µl dd H2O 50 cycles at 95 °C for 15 seconds and 60 °C for 1minute, at 40 ° C for 30 second for cooling were performed (Corney et al 2007).

In order to detect M. haemolytica, PCR mix was prepared by adding 2 µl 10×SYBR Green mix (containing Taq-polymerase), 1µl 25mM MgCl2, 13µl dd H2O and 1µl of each primer for M. haemolytica (10 µmol) and 2 µl (50ng/µl) template DNA. 40 cycles at 95 °C for 10 min and at 95 °C for 15 seconds, at 64 °C annealing for 1 second and at 72 °C elongation for 15 seconds were performed. 10 min at 72 °C final elongation step finalized the reaction.

For melting curve analysis 1 cycle was performed at 95 °C for 0 seconds, at 65 °C for 15 seconds, and continuous reading was performed at 95 °C for 0 seconds and at 0.1 °C for 1 second. Cooling was performed at 40 °C for 30 seconds (Guenther et al 2008)). Real-Time PCR device (Roche Light Cycler L.2.0, Germany) was used.

P. multocida and M. haemolytica strains obtained from Veterinary Control Center Research Institute were used as the positive control. Distilled water was used as a negative control.

Statistical Analysis

The differences between the cell types counted in cytological smears were evaluated with SPSS 11.00 package program by regarding $p<0.05$ as significant.

Results

The numbers of slaughtered cattle in months during a year, the numbers of cattle with pneumonia with the type of pneumonia and the numbers of pasteurellosis and mannheimosis cases detected were listed in Table 2.

A total of 233 cases (7.12%) out of 3270 slaughtered cattle in a year was determined to have some forms of pneumonia in the gross examination. In microscopic examination of the lung samples, 45 (19.31%) fibrinous bronchopneumonias, 80 (34.33%) catarrhal bronchopneumonias, 70 30.04%) interstitial pneumonias, 31 (13.3%) purulent pneumonias, 7

Table 2. Pneumonia types and their monthly distribution in cattle

Months	Number of Slaughter	Number of Pneumonia	*P.multocida**	*M.haemolytica**	Fibrinous	Catarrhal	Interstitial	Purulent	Granulomatous
January	280	18	2	-	4	9	3	2	0
February	270	19	1	-	3	8	4	4	0
March	290	23	1	2	4	8	8	2	1
April	270	17	1	1	3	6	5	3	0
May	260	14	-	-	3	4	5	2	0
June	260	14	1	-	3	4	6	1	0
July	270	12	-	1	3	4	2	2	1
August	280	19	-	-	2	4	6	5	2
September	270	25	2	-	4	8	9	4	0
October	270	26	2	2	4	9	9	3	1
November	280	25	2	2	6	7	9	2	1
December	270	21	1	2	6	9	4	1	1
TOTAL	3270	233	13	10	45	80	70	31	7

* Pneumonia cases identified by Real Time PCR following microbiological isolation

(3%) granulomatous pneumonias were detected. Of the 45 fibrinous bronchopneumonia cases, 23 (51.11%) were diagnosed as pasteurellosis and mannheimosis based on the microbiological and real-time PCR analysis.

In the bacteriological analysis, Pasteurella ssp. was isolated in 23 out of 45 cases (51.11%) with fibrinous bronchopneumonia. The real-time PCR analysis of these cases revealed that 13 were P. multocida (Figure 1) and 10 were M. haemolytica (Figure 2).

In the cytological examination of touch smears prepared from lung samples, epithelial cells with or without cilia, lymphocytes, macrophages, neutrophils, eosinophils and connective tissue cells were observed. Macrophages had mostly large cytoplasm containing intracytoplasmic particles, and their nucleus was generally located on one side of the cell. The mean numbers and standard deviations of the cells counted in touch smears prepared from lung samples were given in Table 3. The number of neutrophils was significantly increased in the lungs with fibrinous bronchopneumonia compared to the other types of pneumonia. Along with the increase in the number of neutrophils in cases diagnosed with pasteurellosis and mannheimosis, it was observed that the numbers of lymphocytes and ciliated epithelium were statistically significantly increased compared to the non-pasteurellosis and non-mannheimosis fibrinous bronchopneumonia cases. Generally, it was observed that there was an increase in macrophages and non-ciliated epithelial cells in catarrhal bronchopneumonia. An increase in the number of lymphocytes in interstitial types of pneumonia was noted. Purulent types of pneumonia were mostly observed with increased numbers of macrophages and neutrophils. In granulomatous pneumonia, macrophages and lymphocytes generally dominated the field with the presence of fewer neutrophils.

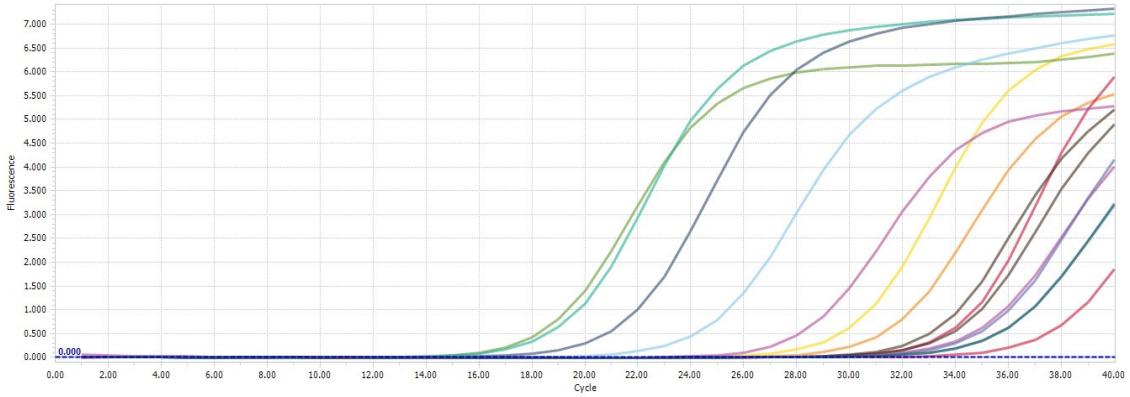

Figure 1. Real-Time PCR graphic of the samples of lungs with fibrinous bronchopneumonia on which the amplification of P. multocida 16S rRNA gene was performed.

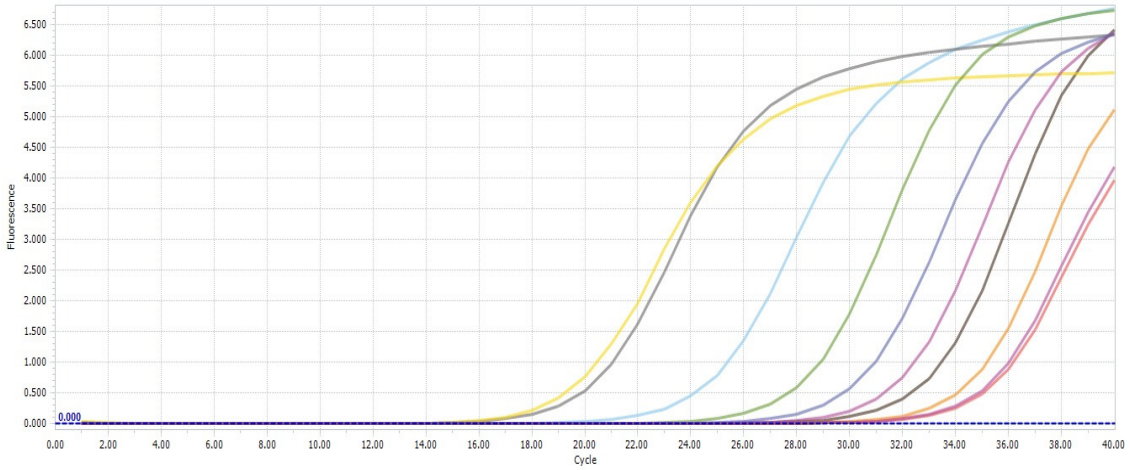

Figure 2. Real-Time PCR graphic of the samples of lungs with fibrinous bronchopneumonia on which the amplification of M. haemolytica sod A gene was performed.

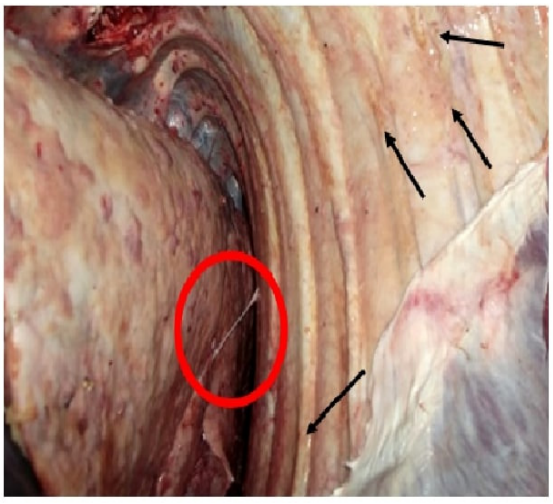

Figure 3. Adherence between the lung and the ribs (red circle), and pleurisy (arrows) in pneumonia caused by P.multocida.

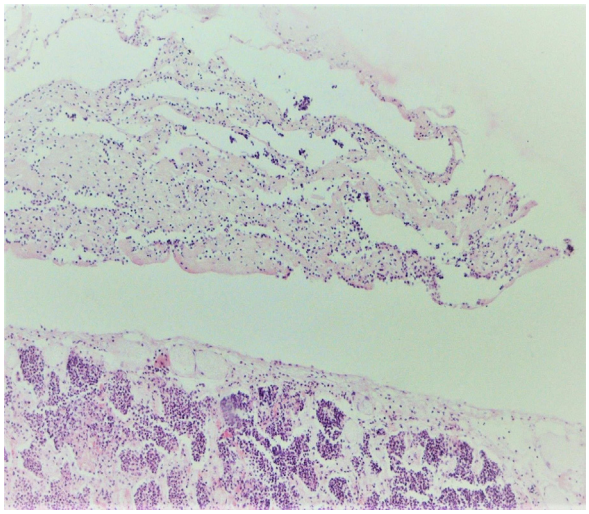

Figure 4. Thickening in the pleura, pleurisy; Pneumonia caused by Pasteurella multocida, hematoxylin and eosin, ×200

Table 3. Mean and standard deviations of cells recorded in smears prepared from lung samples

Type of pneumonia	N	Counted Cell Type						
		Macrophage	Lymphocyte	Eosinophil	Neutrophil	Epithelium With Cilia	Epithelium Without Cilia	Fibrocyte Fibroblast
Fibrinous Bronchopneumonia	45	50.6±5.01^{c}	46.2±4.04^{a}	4.01±0.4^{b}	75.6±5.49^{ef}	5.01±1.41^{a}	3.02±1.21^{cd}	2.3±0.08^{b}
****P.multocida**	13	49.4±4.2^{c}	55.2±1.06^{b}	4.8±0.37^{c}	72.01±4.02^{e}	10.06±1.08^{c}	2.88±0.88^{c}	2.2±0.06^{b}
****M.haemolytica**	10	48.6±4.3^{c}	54.8±1.00^{b}	45,01±0.40^{c}	74.03±4.06^{e}	11.02±1.21^{c}	2.76±0.92^{c}	2.1±0.08^{b}
Catarrhal Bronchopneumonia	80	74.1±4.8^{d}	44.4±2.8^{a}	3.9±0.4^{b}	50.46±5.01^{d}	20.3±3.42^{d}	5.20±1.48^{d}	1.3±0.24^{a}
Interstitial Pneumonia	70	31.8±2.01^{a}	125.2±3.24^{d}	4.08±0.3^{bc}	15.8±0.87^{a}	10.83±3.45^{c}	2.68±0.28^{c}	2.4±0.02^{b}
Purulent Pneumonia	31	72.3±3.2^{d}	52.02±2.6^{ab}	1.00±0.2^{a}	60.22±4.62^{d}	5.3±1.02^{ab}	2.08±0.08^{b}	3.4±0.12^{c}
Granulomatous Pneumonia	7	70.2±5.01^{d}	82.8±3.2^{c}	6.1±0.5^{d}	26.12±4. 4^{c}	8.08±1.02^{c}	1.02±0.28^{a}	4.4±0.82^{d}

*Groups that have different letters in the same column are statistically different p<0,05

** Pneumonia cases identified by real time PCR following microbiological isolation

Table 4. Numbers of cells counted in smears prepared form nasal swab samples

Type of pneumonia	Nasal swab				
	Macrophage(M)	Lymphocyte(L)	Eosinophile(E)	Neutrophil(N)	Epithelium(EP)
Fibrinous broncho-pneumonia	25<M<50	25<L<50	-	N >100	25<EP<50
***P.multocida**	25<M<50	25<L<50	-	N>100	25<EP<50
***M.haemolytica**	25<M<50	25<L<50	-	N>100	25<EP<50
Catarrhal bronchopneumonia	50<M<75	25<L<50	1-3	50<N<75	25<EP<50
Interstitial pneumonia	25<M<50	50<L<75	1-3	25<N<50	25<EP<50
Purulent pneumonia	25<M<50	25<L<50	-	50<N<75	25<EP<50
Granulomatous pneumonia	50<M<75	50<L<75	3-5	25<N<50	25<EP<50

* Pneumonia cases identified by real time PCR following microbiological isolation

Cytological investigations of smears prepared from nasal swabs taken from cattle with pneumonia revealed that the neutrophil number increased in fibrinous bronchopneumonias. There was an increase in the number of macrophages and neutrophils in catarrhal bronchopneumonias, lymphocytes in interstitial types of pneumonia, macrophages, and lymphocytes in granulomatous pneumonia. The types of pneumonia determined and the distribution of relative cell numbers in smears prepared from nasal swabs was given in Table 4.

In the gross examination of lungs with fibrinous bronchopneumonia, infected lung segments, which were dark red to gray in color and viscous to hepatized in tendency, were noted to be separated by a clear border from the healthy tissue. Mostly cranial lobes were affected. Cut surface of these lobes were seen as hepatized areas of pale gray-brown and uniform structures. The lesions in the pleura were quite apparent and there were adherences between the lung and the pleura in some animals (Figure 3). In these cases, a dull and rough appearance was noted along with the thickening in the pleura.

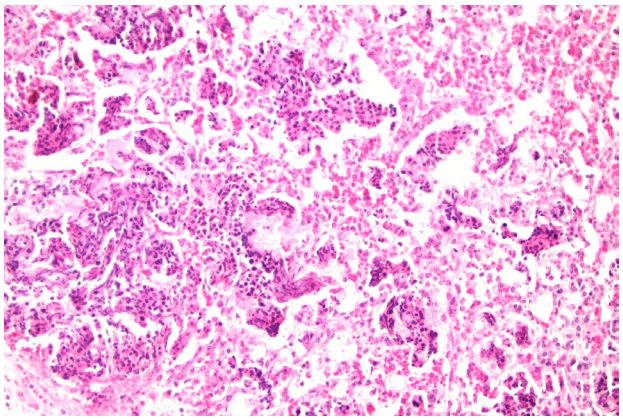

Figure 5. Oat cells (arrows), Pneumonia caused by Pasteurella multocida, hematoxylin and eosin staining, x300

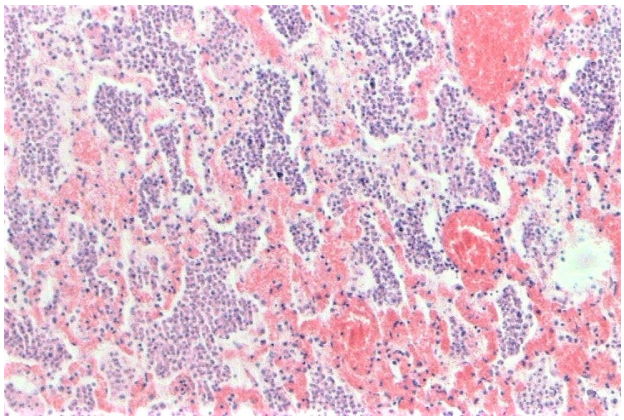

Figure 6. Hyperemia (thick arrow) in the interalveolar septum, edema in the alveolar lumens and neutrophil leukocytes (thin arrow), Pneumonia caused by Mannheimia haemolytica, hematoxylin and eosin staining, x200

The microscopic view of fibrinous pneumonia with pasteurellosis and mannheimosis, which was determined by microbiological and real-time PCR analysis, showed that fibrin strands, edema and alveolar macrophages were intensely accumulated in the alveolar lumens. Necrotic bronchiolitis existed in some bronchiole, and hyperplasia existed in the bronchus-bronchioles epithelium. Thrombosis in lymph capillaries, edema and thickening, pleurisy in interlobular septum (Figure 4) and oat-cells (Figure 5), which are the typical cells seen in pasteurellosis were also noteworthy. Histopathological findings in pneumonia caused by M. haemolytica and P. multocida were similar but oat-cells were less and necrotic areas were more prominent in pneumonia caused by M.haemolytica (Figure 6).

Discussion

Pasteurellosis and mannheimosis are important disease worldwide due to the economic losses it causes in the cattle industry. Regional distribution of the disease is therefore important for the local breeders. In this study, the prevalence of the disease in the city of Sivas, Turkey was investigated by conventional and real-time PCR analysis in nasal swab and lung samples. Detailed pathological and cytological examinations were also performed to determine the course of the disease as well as the type of pneumonia seen.

The ratio of cattle pneumonia varies greatly among the studies. Haritani et al. (1990) reported that pneumonia could reach to 65.83% among calves. In large-scale investigations, pneumonia ratios were reported to be between 3.89% and 20.1% in cattle (Alexander et al 1989, Maity and Deb 1991, Ozen et al 2009, Oruc 2011). In the present investigation, pneumonia was detected in 9.05%. This ratio is within the limits of the ratios reported previously. In the current investigation, pasteurellosis and mannheimosis were also detected in 51.11% of the fibrinous bronchopneumonia and 9.87% of the total cases of pneumonia.

It has been stated that the results obtained by the methods used in many studies for determining the agents of pneumonia in cattle do not exactly the same. Therefore, in this study, cytological findings reported include lung tissues only determined microbiologically Pasteurella ssp. and identified as M.haemolytica and P.multocida by Real Time PCR.

The seasonal distribution of pneumonia cases is an important issue since the measures to be taken can be optimized to reduce the occurrence of respiratory diseases. In a previous study, cases of pneumonia according to seasons were recorded as 16.0% in summer, 21.7 in rainy seasons and 23.0% in winter (Maity and Deb 1991). In the same study, the highest rate was in November (27.7%) and the lowest rate in June (13.9%). The results of the current investigation are consistent with the findings of the literature. The incidence rate of pneumonia was observed to be highest during the cold winter months of the year. This result indicates that the severe weather conditions in Sivas may play a role in the development of pneumonia as a predisposing factor. These results demonstrate that it is crucial to take good heating measures and to consider the best time planning for vaccination against pasteurellosis and mannheimosis.

Gross examination of lungs with fibrinous pneumonia in this study showed gray-brown-red hepatized areas separated by clear-cut boundaries. In many of these cases, pleura was also thickened with a dull and rough appearance. Cross-sections of lungs were mottled-looking. In microscopic examinations of cases diagnosed with pasteurellosis and mannheimosis alveolar macrophages, edema and fibrin strands in the alveolar lumens were noteworthy. Necrotic bronchiolitis was observed in some bronchioles, and hyperplasia was observed in the bronchus-bronchioles epithelium. Thrombosis in lymph capillaries and the presence of oat-cells, which have characteristic elongated and rounded nuclei were the commonly recorded observations. These findings were generally in compliance with the results of similar studies described pas-

teurellosis and mannheimosis (Hazıroglu et al 1997, Ozen et al 2009, Lopez and Martinson 2017).

Careful examination of smears prepared from nasal swabs and lung samples can reveal important results in determining the type of pneumonia (Oruc 2011, Van Leenen et al 2019). In cytological examinations, the type and proportion of inflammatory cells are therefore important. It was stated that significant increases in the numbers of neutrophils, eosinophils, macrophages, and lymphocytes occur in cases of pneumonia (Weiss et al 1991, Oruc and Tuzcu 2010, Oruc 2011). In the present study, the number of neutrophils was seen to increase in cases with fibrinous bronchopneumonia compared to the other types of pneumonia. The number of lymphocytes and ciliated epithelium, in addition to neutrophils, was noted to increase in cases diagnosed with pasteurellosis and mannheimosis. Specific inflammatory cellular increases were also recorded with other types of pneumonia. These findings suggest that the smears prepared from nasal swabs might give clues in the diagnosis of pneumonia types and even the pasteurellosis and mannheimosis when the animals are still alive. In this way, proper treatments can also be applied to reduce potential losses.

The bacteriological culture method is a commonly used standard technique in the diagnosis of pasteurella agents (Collins et al 1995). However, the method is time-consuming and troublesome. In addition, due to mix infections, pasteurella agents can be overlooked and hence incorrect results can be reached. Antigen-antibody binding based tests can also be commonly used in the diagnosis of some bacterial and viral diseases. However, cross-reactions may also be seen in these tests and therefore their reliability is in question. Real-time PCR analysis is reliably used in the diagnosis of many bacterial agents (Probert et al 2004, Mekkes and Feberwee 2005). Optimized PCR conditions can further increase the reliability. In the current investigation, the technique was proven to be useful in the diagnosis of P. multocida and M. haemolytica in cattle with pneumonia.

The results of the present study showed that pasteurellosis and mannheimosis constitutes 9.87% of the total amount of pneumonia detected in cattle and the rate was especially significant among the fibrinous types of pneumonia in which 51.11% is pasteurellosis and mannheimosis. The disease is known to be widespread in the world and our findings confer the results recorded for other regions of Turkey (Ozer 1985, Ozturk et al 1996, Gündüz and Erganis 1998, Ozen et al 2009).

Conclusion

In conclusion, cytological, histopathological, microbiological, and molecular techniques were used in the diagnosis and characterization of pasteurellosis and mannheimosis in cattle slaughtered in Sivas. The prevalence of the disease was seen to be in the range reported by previous studies. Cytological features of smears prepared from nasal swabs and lung samples were identified and its use in differentiating the type of pneumonia was discussed. It was also concluded the real-time PCR analysis with optimized conditions could be used as a fast and reliable technique in the diagnosis of pasteurellosis and mannheimosis in cattle.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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References

Akıllı H, Yoldaş A, Özmen M, Topçuoğlu H, et al., 2012. Çukurova yöresinde yığırlarda görülen granülomatöz pnömonilerin etiyolojisinin histopatolojik ve moleküler yöntemlerle belirlenmesi. AVKAE Derg, 2,1-6.

Alexander BH, MacVean DW, Salman MD, 1989. Risk factors for lower respiratory tract disease in a cohort of feedlot cattle. J Am Vet Med Assoc, 195, 207-211.

Altun S, 2015. Pnömonili Sığır akciğerlerinde Mycoplasma bovis enfeksiyonunun patolojik ve moleküler yöntemlerle araştırılması. Sağlık Bilimleri Enstitüsü, Veteriner Patoloji Anabilim Dalı, Doktara Tezi, Erzurum: Atatürk Üniversitesi, 2015.

Amrine DE, White BJ, Larson RL, Mosier DA, 2014. Pulmonary lesions and clinical disease response to Mannheimia haemolytica challenge 10 days following administration of tildipirosin or tulathromycin. J Anim Sci, 92(1), 311–319.

Arda M, Minbay A, Leloğlu N, Aydın N, et al., 1999. Özel Mikrobiyoloji, In:Pasteurellaceae Familyası , Ed;Aydın N, Fifth edition, Medisan, Ankara, Türkiye, pp:64-74.

Batmaz H. Pasteurella pneumonileri ve ekonomik önemi. Sığırların Solunum Sistemi Hastalıkları Sempozyumu, Bursa, 2006, Uludağ Üniversitesi, Veteriner Fakültesi, TÜBİTAK: 58-64.

Caswell JL and Williams KJ, 2007. Respiratory system. In: Jubb (ed). Kennedy and Palmer's Pathology of Domestic Animals, 5st ed.523-629.

Collins CH, Layne PM, Grange JM, 1995. Microbiological Methods, In: Actinobacillus, Pasteurella, Yersinia, Cardiobacterium and Francisella, Seventh Edition, Butterworth- Heinemann Ltd, London, Great Britain, 343-349.

Corney BG, DialloI S, Wright LL, Hewitson GR, et al., 2007. Pasteurella multocida detection by 5′ Taq nuclease assay: A new tool foruse in diagnosing fowl cholera. J Microbiol

Methods, 69, 376–380.

Çiftçi MK, Ortatatlı M, Erer H, Hatipoğlu F, et al., 2015. Veteriner Sistemik Patoloji, 1. Cilt. Selçuk Üniversitesi Basımevi, Konya, Türkiye, pp;157.

Erbaş G, Kaya O, 2008. Aydın ve İzmir bölgesindeki sığırlardan Pasteurella multocida'nın izolasyonu, tiplendirilmesi ve antibiyotiklere duyarlılıkları. Bornova Vet Kont Araşt Enst Derg, 44, 7-14.

Frank GH, 1986. The role of Pasteurella haemolytica in the bovine respiratory disease complex. Vet Med, 12, 841-846.

Guenther S, Schierack P, Grobbel M, Lübke-Becker A, et al.,2008. Real-time PCR assay for the detection of species of the genus Mannheimia. J Microbiol Methods, 75, 75–80.

Gündüz K, Erganiş O, 1998. Pnömonili sığır akciğerlerinden izole edilen Pasteurella haemolytica suşlarının biyotiplendirilmesi ve serotiplendirilmesi. Veterinarium, 9, 11-19.

Haritani M, Nakazawa M, Hashimoto K, Narita M, et al., 1990. Immunoperoxidase evaluation of the relationship between necrotic lesions and causative bacteria in lungs of calves with naturally acquired pneumonia. Am. J Vet Res, 12, 1975-1979.

Hazıroğlu R, Erdeğer J, Gülbahar MY, Kul O,1997. Association of Pasteurella haemolytica, Pasteurella multocida and Haemophilus somnus with pneumonia in calves. Dtsch Tierarztl Wschr, 104, 125-164.

Kamp EM, Bokken GCAM, Vermeulen TMM, de Jong MF, et al,.1996. A specific and sensitive PCR assay suitable for large-scale detection of toxigenic Pasteurella multocida in nasal and tonsillar swabs specimens of pigs. J Vet Diagn Invest, 8, 304-309.

Kılıç A, Muz A, 2004. Pnömonili sığır akciğerlerinden bakteri izolasyonları ve izole Pasteurella'ların polimeraz zincir reaksiyonu ile saptanması, Türk Anim Sci, 28, 217-223.

Lichtensteiger CA, Steenbergen SM, Lee RM, Polson DD, et al.,1996. PCR analysis for toxigenic Pasteurella multocida. J Clin Microbiol, 34, 3035-3039.

Lopez A, Martinson AS., 2017. Pathologic Basis of Veterinay Disease, In: Respiratory System, Mediastinum and Pleurea, Sixth Edition, St.Louis, Missouri, China, pp: 471-560.

Maity B, Deb P,1991. Seasonal variation in incidence of pneumonia in cattle. Ind J Anim Sci, 61, 261-262.

Mekkes DR, Feberwee A, 2005. Real-time polymerase chain reaction for the qualitative and quantitative detection of Mycoplasma gallisepticum. Avian Pathol, 34, 348-354.

Oruç E, Tuzcu M, 2010. Veteriner Sitoloji. Adana, Türkiye: Net Kopya Basımevi.

Oruç E, 2011. Cytopathologic studies in sheep pneumonias. Eurasian J Vet Sci, 1, 67 71.

Özen H, Karaman M, Şahin M, Özcan K, 2009. PCR Detection of Mycoplasma bovis, M. dispar, M. bovirhinis and M. mycoides subsp. mycoides (small colony type) and investigations of pathological findings in pneumonic cattle. Kafkas Üniv Vet Fak Derg,15, 125-133.

Özer H, 1985. Besi danalarında exudative pnömonilerin yayılışı. Elazığ Bölgesi Vet Hek Odası Derg, 3, 63-70.

Öztürk G, Özcan C, Kalender H, 1996. Elazığ Et Balık Kurumu mezbahasında kesilen sığırlarda rastlanan pnömonilerin patolojik ve bakteriyolojik olarak incelenmesi. Pendik Vet Mikrobiyol Derg, 27, 163-174.

Probert WS, Schrader KN, Khuong NY, Bystrom SL,et al., 2004. Real-time multiplex PCR assay for detection of Brucella spp., B. abortus, and B. melitensis. J Clin Microbiol, 42, 1290-1293.

Quinn PJ, Markey BK, Leonard FC, Fitzpatrick ES, et al., 2016. Concise Review of Veterinary Microbiology, 2nd ed. Wiley-Blackwell, UK.

Van Leenen K, Van Driessche L, De Cremer L, Gille L, et al., 2019. Factors associated with lung cytology as obtained by non-endoscopic broncho-alveolar lavage in group-housed calves. BMC Vet Res, 15, 167.

Weiss DJ, Bauer MC, Whiteley LO, Maheswaran SK, et al., 1991. Changes in blood and bronchoalveoler lavage fluid components in calves with experimentally induced pneumonic pasteurellasis. Am. J. Vet. Res, 52(2), 337-344.

Yates WDG, 1982. A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. Can J Comp Med, 46, 225-263.

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Ethical Approval

The data and information presented in this article were obtained within the framework of academic and ethical rules. Ethical declaration that the evaluation results were in accordance with scientific and ethic rules, was received from the authors.

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