



CASE REPORT

Pioneer Study on Isolation of *P. multocida* from Cat Pyothorax in India

Manju Soman^{1*}, Sreeja R Nair¹, C Divya² and M Mini¹

¹Department of Veterinary Microbiology, ²Department of Veterinary Pathology, College of Veterinary and Animal sciences, Mannuthy, Thrissur

ARTICLE INFO

Received: September 15, 2013

Revised: October 09, 2013

Accepted: January 18, 2014

Key words:

Antibiogram

Cat

Histopathology

Pasteurella multocida

PCR

PM

Pyothorax

*Corresponding Author

Manju Soman

soman_manju@yahoo.com

ABSTRACT

Respiratory infections are common in feline population throughout the world. However systematic reports on the various disease conditions affecting the respiratory system of felines are scanty. A detailed pathological and microbiological investigation into a case of pyothorax observed in a 2 year old cat is reported herein. The carcass of a cat aged two years brought for post-mortem examination with the history of dyspnea formed the study material. Post mortem examination revealed gross lesions of fibrinopurulent pleurisy, cardiac hypertrophy, splenic congestion, metastatic suppurative hepatitis, nephrosis and congestion of brain. Histological examination of the representative tissue samples revealed fibrinonecrotic pleuritis, alveolar collapse, inflammatory cell infiltration and alveolar epithelialization within the lung parenchyma. Gram negative, coccobacillary organisms suggestive of the genus *Pasteurella* was isolated from the pleural cavity. The organism was identified as *Pasteurella multocida* based on biochemical characteristics, mice pathogenicity and PM-PCR. The antibiogram of the isolate revealed sensitivity to Ciprofloxacin, Chloramphenicol, Ceftriaxone, Amoxicillin and Cotrimoxazole and resistance to Gentamicin. This is the first report of isolation of *P. multocida* from a case of pyothorax in cat from India.

Cite This Article as: Soman M, Nair S R, Divya C and Mini M, 2014. Pioneer study on isolation of *P. multocida* from cat pyothorax in India. Inter J Vet Sci, 3(1): 7-10. www.ijvets.com

INTRODUCTION

Pyothorax or pleural empyema is a condition characterized by the accumulation of purulent material in the pleural cavity. This can occur as a sequel to an infection from a penetrating wound or as an extension of bacterial pneumonia or through haematogenous spread. It has been found that majority of the infections are caused by a mixture of obligate anaerobic and facultatively anaerobic bacteria (Walker *et al.*, 2000). Studies reveal that isolates from lesions of pyothorax in cats are consistent with the normal oropharyngeal flora of the animal (Barrs *et al.*, 2005). *Pasteurella multocida*, which are commensals of the oropharynx and upper respiratory tract of a wide spectrum of animals (Parija, S .C. 2009), have been identified as one of the common bacteria causing infection in cats (Walker *et al.*, 2000).

The present paper documents the isolation and identification of *Pasteurella multocida* from a case of pyothorax in cat and its sensitivity to the commonly used antibiotics. A detailed pathological and microbiological investigation into the case is reported herein.

MATERIALS AND METHODS

Carcass of a 2 year old cat, with a history of dyspnoea, lethargy and anorexia was presented at the College of Veterinary and Animal Sciences, Mannuthy. A detailed necropsy was carried out and the gross lesions recorded. Smears were made from the purulent exudate present in pleural cavity and subjected to Wright's and Gram's staining protocols. The exudate was collected aseptically for cultural isolation of the causative organism. Representative tissue samples were collected and fixed in freshly prepared 10% neutral buffered formalin. The samples were processed using standard protocols, embedded in paraffin and sections were cut at 5µ thickness. The sections were stained with routine Haematoxylin and Eosin (Bancroft and Gamble, 2008) to evaluate the various histopathological alterations.

Purulent exudate from the pleural cavity of the cat was inoculated on Brain Heart Infusion Agar (BHIA), 10% bovine Blood Agar and MacConkey Agar and incubated for 24 hours at 37°C. The sample was also inoculated on Sabouraud Dextrose Agar (SDA) and

incubated at room temperature for 7 days. Characterization of the isolate was done based on morphology, cultural characters and biochemical tests such as IMViC, Nitrate reduction, Urease test and TSI test as per Quinn *et al.* (2002).

Pathogenicity testing was done in one month old Swiss albino mice as per Curtis (1985). The mice were observed for signs of infection and death. Heart blood smears and impression smears prepared from liver and spleen of the dead mice were stained with Leishman's stain and examined microscopically. Re isolation of the organism from heart, liver, spleen, kidney and lungs was tried in 10% bovine blood agar.

Confirmation of the identity of the isolate was done by the *Pasteurella multocida* specific Polymerase chain reaction (PM-PCR) assay as per Townsend *et al.* (1998) with minor modifications. Template DNA was extracted by the method of heat lysis of the bacterial culture. PM-PCR was carried out in an Applied Biosystems thermal cycler using *P. multocida* species specific primers KMT1SP6 and KMT1T7 designed to amplify the KMT1 gene with an amplicon size of 460bp unique to *P. multocida*.

The antibiogram was carried out as per Bauer *et al.* (1966) against 6 antibiotics namely Ciprofloxacin, Chloramphenicol, Ceftriaxone, Amoxicillin, Cotrimaxazole and gentamicin.

RESULTS

Upon necropsy, the thoracic cavity was found filled with fibrinopurulent exudate. The exudate filled the space between parietal and visceral pleura as well as extended towards the surface of lung. Pleura adhesion and collapse of lung were discernible. Cardiac hypertrophy and congestion of spleen could be noticed. Focal white areas could be observed on the capsular surface of liver suggestive of metastatic suppurative hepatitis. Gastrointestinal tract was found empty. Cerebral congestion and nephrosis were the other lesions observed.

Fluid obtained from the pleural cavity was observed to be turbid with slight yellow colouration. Microscopic examination of smear from the exudate revealed presence of numerous gram positive cocci (arranged in linear fashion suggestive of streptococci – fig.1) together with huge population of gram negative coccobacillary organisms. Degenerated neutrophils (fig.1) were present in abundance.

Upon histological examination, pleural surface of lung was found covered with necrotic debris and inflammatory cells predominated by neutrophils. Membranous diphtheritic deposit over the pleura was noticeable (fig.2). Alveolar necrosis and inflammatory cells persisted to a short distance from the pleural surface towards the lung parenchyma beyond which alveoli were collapsed accompanied by thickening of alveolar wall suggestive of alveolar epithelialization (fig.3). Overall histopathologic observation of lung and pleura could be assumed as suppurative pleuritis.

Microscopic examination of liver and kidney revealed changes consequent to tissue anoxia. Diffuse paracentral necrosis and individualization of hepatocytes could be observed in liver parenchyma (fig.4). Kidney revealed

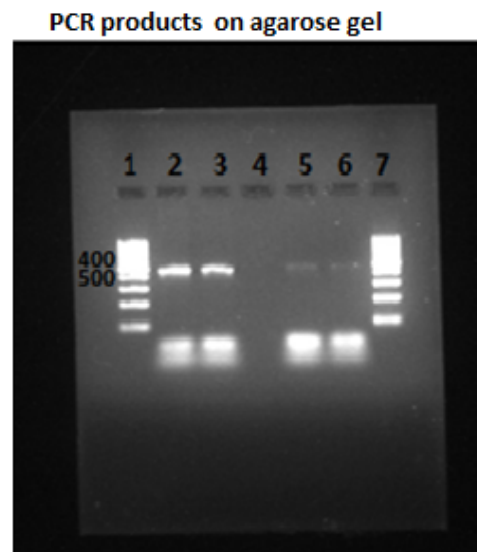
generalized cloudy swelling and vacuolar degeneration of the tubular epithelial cells (fig.5). Tubular lumen was blocked by enlarged lining cells. Histologic section of spleen presented abundance of white pulp with accessory germinal centres indicative of reactive spleen (fig.6).

Following 24 hours of incubation, the BHIA plates inoculated with the pus material yielded small round colonies while the blood agar plates presented smooth, convex, translucent, non hemolytic colonies with a sweetish odor. Gram's staining of colonies from both the plates revealed small, Gram negative, coccobacillary organisms suggestive of the genus *Pasteurella*. MacConkey agar and SDA failed to yield any growth.

The organism was found to be non motile, and catalase and oxidase positive. Results of biochemical tests showed that the isolate was positive for indole, but negative for Methyl Red, Voges-Proskauer, Citrate, Nitrate and Urease tests. Inoculation on TSI slant showed the reaction Yellow slant, Yellow butt, no gas and no H₂S production.

The mice subjected to pathogenicity testing, with the live isolate, died in six hours while the control mice remained alive. The gross lesions observed on postmortem were petechial hemorrhage of liver and heart, general congestion of all organs and fluid accumulation in peritoneal cavity. Heart blood smears and impression smears from liver and spleen on Leishman's staining revealed bipolar stained organisms. *Pasteurella multocida* was re isolated from heart, liver, spleen, kidney and lungs of the dead mice on 10% bovine blood agar and its identity was confirmed by PM-PCR.

Agarose gel electrophoresis of the amplified PCR product revealed an amplicon size of 460bp when viewed under gel documentation system. Thus the isolate was confirmed as *P. multocida* and was named as CPI.



1.7-DNA marker; 2.3-CPI; 5.6-positive control; 4-negative control

Antibiotic sensitivity tests revealed that the isolate showed sensitivity to Ciprofloxacin, Chloramphenicol, Ceftriaxone, Amoxicillin and Cotrimoxazole and resistance to Gentamicin.

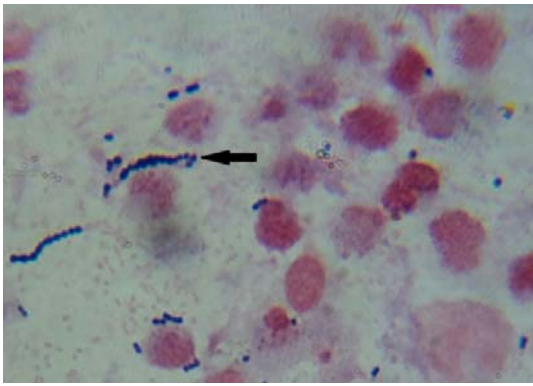


Fig.1: Gram positive cocci (Gram's X1000)

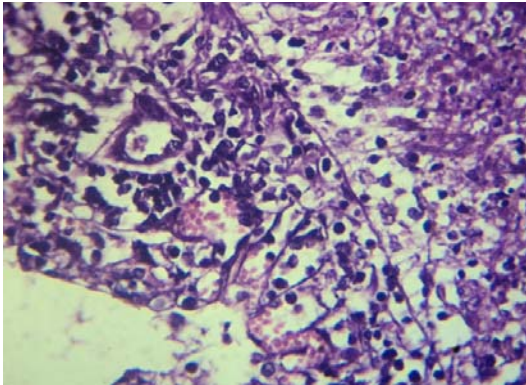


Fig. 2: Lung - Pleural adhesion (H&E X400)

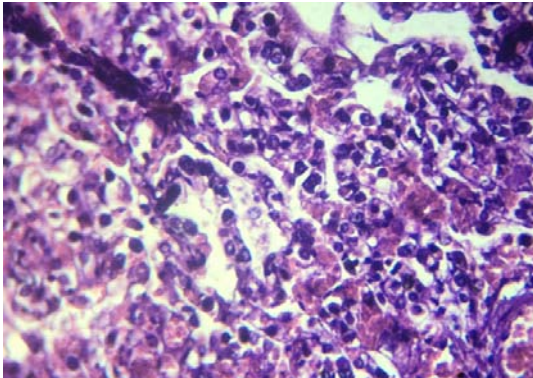


Fig. 3: Alveolar collapse & epithelialization (H&E X400)

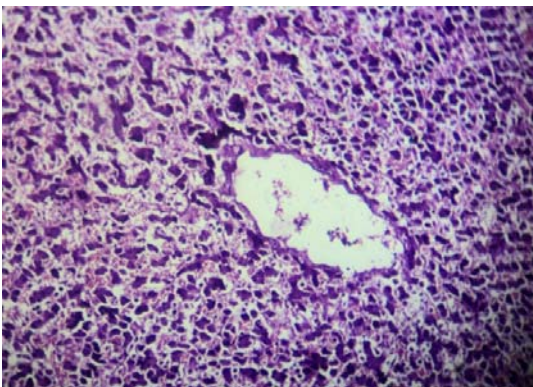


Fig. 4: Liver - Paracentral necrosis & individualization of hepatocytes (H&E X400)

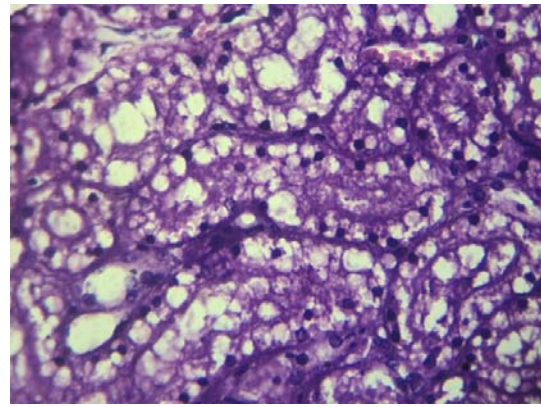


Fig. 5: Kidney-Vacuolar degeneration (H&E X400)

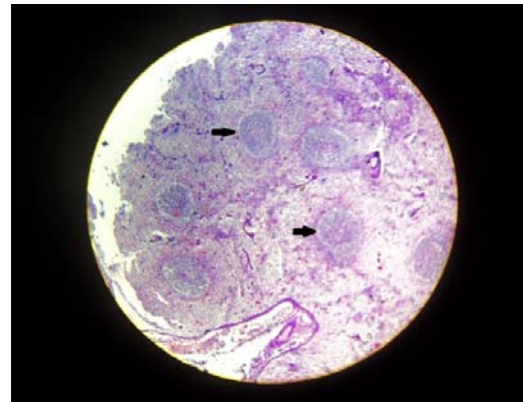


Fig. 6: Reactive spleen H&E X 40)

DISCUSSION

Respiratory infections are not uncommon in cats, but rarely diagnosed. Cats with infectious pneumonia may lack clinical signs and have unremarkable results for a CBC and thoracic radiography, yet frequently have systemic infections as reported by Macdonald *et al.*, 2003. Alveolar epithelialization due to naturally occurring virulent systemic feline calicivirus infection in cats has been reported by Pesavento in 2004. Since epithelialization and reactive spleen which is suggestive of viral etiology is present, detailed examination for identification of viral etiology is warranted in similar cases. Significant understanding of the pathology of various respiratory infections affecting the feline population will undoubtedly play a major role in the control of this ubiquitous, sometimes fatal, often exasperating, and always troublesome problem, so that it would never turn out to be a menace to the feline world. Primary lesion observed in the present case was suppurative pleuritis which led to pulmonary collapse. Hepatic and renal lesions might be consequent to tissue anoxia which in turn is sequel to pulmonary collapse. Since the primary focus of infection is pleura without predominant involvement of lung, the route of entry is possibly not the upper respiratory tract. The gross and histopathologic lesions observed in thoracic cavity in the present case were in agreement with that observed by Stevenson *et al.*, 1973, who reported pleurisy in concurrence with peritonitis in a cat.

Reports on the infectious etiology of pleural empyema in cats are very few. Love *et al.* (1982) and Ottenjann *et al.* (2008) detected *P. multocida* as the most frequently isolated facultative anaerobic organism in cases of cat pyothorax. Mohan *et al.* (1997) carried out detailed characterization of *Pasteurella* isolates obtained from conditions of pyothorax and other respiratory infections in cats and dogs and recorded a preponderance of *P. multocida* subspecies *multocida* and *septica*. Of this most of the serotypes of *P. multocida* belonged to capsular type A, except for a solitary isolate from a cat which was capsular type D.

The PM-PCR assay developed by Townsend *et al.* (1998) detects all the subspecies of *P. multocida*. In this study, species specific primers KMT1SP6 and KMT1T7 could amplify the 460 bp fragment within the KMT1 gene of the isolate DNA which is specific for *P. multocida*. Hence the isolate was confirmed as *P. multocida*.

Boyle *et al.* (2005) reported that cats with pyothorax could survive if subjected to early treatment with proper choice of antibiotics. All *Pasteurella* isolates obtained from cases of pyothorax in cats in his study were found to be susceptible to Amikacin, Ampicillin, Amoxicillin clavulanate, ceftriaxone, enrofloxacin, gentamicin, tetracycline and sulphamethoxazole-trimethoprim. In this study, the isolate was found to be sensitive to Ciprofloxacin, Chloramphenicol, Ceftriaxone, Amoxicillin and Co-trimoxazole, but resistant to Gentamicin.

Conclusion

This is a pioneer work on feline pyothorax in India. Identification of *P. multocida* as a causative agent of pyothorax in cat lays the foundation for future more elaborate studies on the infectious etiology of feline pyothorax.

Acknowledgement

The authors are highly thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, for providing all kind of facilities.

REFERENCES

- Bancroft JD and M Gamble, 2008. Theory and Practice of Histological Techniques. 6th ed. Churchill Livingstone, United States of America, 725p.
- Barrs VR, GS Allan, P Martin, JA Beatty and R Malik, 2005. Feline pyothorax: a retrospective study of 27 cases in Australia. J Feline Med Surgery, 7: 211-222.
- Bauer AW, WM Kirby, JC Sherris and M Turck, 1966. Antibiotic susceptibility testing by a standardized single disc method. Amer J Clin Pathol, 45: 493-496.
- Boyle TE and EC Hawkins, 2005. Feline Pyothorax. Standards of care: emergency and critical care medicine, 7: 7-12.
- Curtis PE, 1985. Pasteurella multocida In: C. H. Collins and JM Grange (editors), Isolation and Identification of Microorganisms of Veterinary Importance. Academic Press, London, P-43.
- Love DN, RF Jones, M Bailey, RS Johnson and N Gamble, 1982. Isolation and characterisation of bacteria from pyothorax (empyema) in cats. Vet Microbiol, 7: 455-61.
- Macdonald ES, CR Norris, RB Berghaus and SM Griffey, 2003. Clinicopathologic and radiographic features and etiologic agents in cats with histologically confirmed infectious pneumonia: 39 cases (1991-2000). J Am Vet Med Assoc, 223: 1142-1150.
- Mohan K, PJ Kelly, FW Hill, P Muvavarirwa and A Pawandiwa, 1997. Phenotype and serotype of Pasteurella multocida isolates from diseases of dogs and cats in Zimbabwe. Comparative Immunol Microbiol Infectious Dis, 20: 29-34.
- Ottenjann M, A Lübke-Becker, H Linzmann, L Brunner and B Kohn, 2008. Pyothorax in 26 cats: clinical signs, laboratory results and therapy (2000-2007). Berl Munch Tierarztl Wochenschr, 121: 365-73.
- Parija SC, 2012. Textbook of Microbiology and Immunology. 2nd edition. Elsevier Health sciences, India.
- Pesavento PA, NJ Maclachlan. L Dillard-Telm, CK Grant and KF Hurley, 2004. Pathologic, Immunohistochemical and Electron Microscopic Findings in Naturally Occurring Virulent Systemic Feline Calicivirus Infection in Cats. Vet Path, 41: 257-263.
- Quinn PJ, BK Markey, ME Carter, WJ Donnelly and FC Leonard, 2002. Veterinary Microbiology and Microbial Diseases. Blackwell Sciences Ltd, 48-57, 254-258.
- Stevenson RG, SE Tilt and JG Purdy, 1971. Feline infectious peritonitis and pleurisy. Can Vet J, 12: 97-99.
- Townsend KM, AJ Frost, CW Lu, JM Papadimitriou and HT Dawkins, 1998. Development of PCR assays for species and type specific identification of *P. multocida* isolates. J Clin Microbiol, 36: 1096-1100.
- Walker AL, SS Jang and DC Hirsh, 2000. Bacteria associated with pyothorax of dogs and cats: 98 cases (1989-1998). J Amer Vet Med Assoc, 216: 359-63.