International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 7(B); July 2018; Page No. 13953-13955
DOI: http://dx.doi.org/10.24327/ijcar.2018.13955.2511

Case Study

MOLECULAR CONFIRMATION AND THERAPEUTIC MANAGEMENT OF HAEMOTROPIC MYCOPLASMOSIS IN A DOG, A CASE STUDY

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A R T I C L E I N F O

Article History:

Received 10th April, 2018
Received in revised form 18th May, 2018 Accepted 26th June, 2018
Published online 28th July, 2018

Key words:

Mycoplasma haemocanis, PCR, Treatment

A B S T R A C T

Mycoplasma haemocanis (formerly known as Haemobartonella canis) is the cause of haemotropic mycoplasmosis in dogs. The parasites attach to the surface of the red blood cell, and have the potential to cause severe alterations of the cell's shape, resulting in anaemia.

A three year old Rottweiler dog was presented to the University Veterinary Hospital with symptoms of lethargy and reduced appetite. Upon clinical examination pyrexia (103º – 105º F), pale mucous membranes and lymphadenopathy were noticed. Blood smear examination revealed the presence of small coccoid organisms in the periphery of the RBC suggestive of haemoplasma. Presence of Mycoplasma hemocanis was confirmed using PCR. Hematobiochemical alterations included microcytic anaemia, granulocytosis, and thrombocytopenia, decreased haematocrit values along with hypoalbuminemia and elevated liver enzymes. Animal was successfully treated with parental administration of oxytetracycline and prednisolone along with supportive therapy.

INTRODUCTION

Haemotropic Mycoplasmas or haemoplasmas have been characterized as cell wall-deficient, uncultivable bacteria that colonize the outside of erythrocytes and infect a wide range of vertebrate hosts. The organism is considered as pleomorphic and it can be noticed as rod-shaped, spherical, or ring-shaped and are found individually or in chains across the red cell surface (Wengi *et al*., 2008). Haemotropic mycoplasmas were previously known as *Haemobartonella* and *Eperythrozoon*, but now are reclassified within the genus *Mycoplasma* according to 16S rRNA gene sequencing (Messick 2004). The first documented occurrence of mycoplasmas in dogs was in 1934. Mycoplasma organisms have not been successfully grown by in vitro culture; therefore, prior to the advent of PCR testing hemoplasmas were only identified by light microscopy during cytological evaluation of blood smears. The organism has got worldwide prevalence and arthropod vectors are considered to be the important mode of transmission (Roura *et al.,* 2010). However the infection can also spreads through the fight between dogs, and also during blood transfusion. Molecular detection and successful therapeutic management of haemotropic mycoplasmosis in a three year old Rottweiler dog is described in this article.

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MATERIALS AND METHODS

A three year old male Rottweiler was presented to the university veterinary hospital with the symptoms of lethargy and reduced appetite. Detailed clinical examination was done and findings were documented. Smears were stained with Giemsa stain and examined. Whole blood was collected in 2ml anti-coagulant coated vial and haematological parameters were estimated, including RBC, WBC and platelet count (PLT), HGB concentration. Serum was separated from the collected blood and biochemical parameters such as Creatinine, Blood urea nitrogen, Alanine aminotransferase, Total protein, albumin and A:G ratio were estimated.

Anti-coagulant blood was used for DNA extraction, the extraction was done using commercial DNA extraction kit (Qiagen blood and tissue kit). As per the prescribed protocol. Quality (absorbance ratio 260/280) was measured spectroscopically (Nanodrop, Thermo Scientific, Fisher, USA) for extracted sample. Extracted DNA sample was eluted in nuclease-free water and stored at−20 ◦C until use. The PCR was performed on the extracted DNA using species specific primers.

The amplification was carried out in 25µl reaction mixture containing 12.5µl EmeraldAmp® GT PCR Master Mix, made Takara 7.5µl of nucleus free water, 1µl of each primers and 3µl of template. The positive DNA of *Mycoplasma haemocanis* obtained from Dr SeverineTasker, School of Veterinary Sciences, Bristol University, U.K. was used as positive control for the above PCR reaction.

The cycling was carried out in MJ mini personal thermal cycler (BIO RAD) as per protocol mentioned in table 1. The primers used were 5' GAAACTAAGGCCATAAATGACGC 3' as forward and 5'ACCTGTCACCTCGATAACCTCTA 3' as reverse respectively.

Table 1 PCR protocol

No.	PCR	Temperature	Time	
1.	Initial denaturation	98ºC	3 min	
2.	Denaturation	98ºC	10sec	32cycles
3.	Annealing	63ºC	30sec	
4.	Extension	72ºC	1min	
5.	Final extension	72ºC	10min	

RESULT

The major clinical signs observed in the present study are blanched mucous membrane due to severe anaemia, enlarged lymph nodes, and pyrexia. Peripheral blood smear examination revealed presence of small coccoid organism in the periphery of the RBCs which was suggestive of Haemotropic mycoplasmosis (Fig. 1). No other blood parasites could be noticed in the stained blood smear.

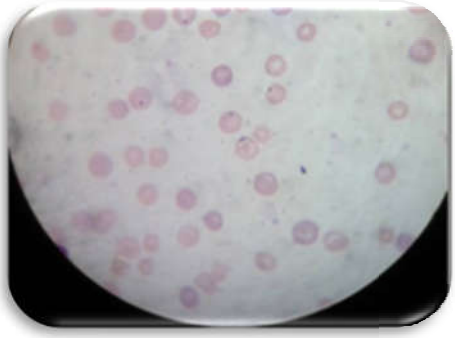

Fig 1 Small coccoid organism in the periphery of RBC

Haematological analysis revealed mild leucocytosis with increase in granulocytes along with anaemia and thrombocytopenia.

Table 2 Hematological parameters

Parameters	Unit	Result	Normal range
WBC (10^3/µl)		18.0	6.0-17.0
Lymphocytes (10^3/µl)		3.4	0.7-5.1
Monocytes (10^3/µl)		0.9	0.2-1.7
Granulocytes (10^3/µl)		13.7	4.4-12.6
RBC (10^6/µl)		3.26	5.50-8.50
HGB	(g/dl)	8.4	12-18
VPRC	(%)	30.3	37-55
Platelets	(10^3/µl)	60	160-525

Table 3 Biochemical parameters

Parameters	Unit	Result	Normal range
Protein	g\dl	7.3	5.4 – 7.5
Albumin	g/dl	1.8	2.3 – 3.1
Globulin	g\dl	5.5	2.4 – 4.4
ALT/SGPT	U/L	128	10 - 109
Bilirubin	mg/dl	0	0 – 0.3
Creatinine	mg/dl	1.02	0.5 – 1.7

The PCR reactions using species specific PCR targeting 16S rRNA gene of *Mycoplasma haemocanis* yielded amplicon of size 300bp (Fig. 2.). No product was amplified in the negativecontrol.

Fig 2 PCR amplification of Haemotropic mycoplasmosis

Treatment

The animal was treated with intravenous administration of oxytetracycline injection @ 10mg/kg body weight for 5 days and intra muscular injection of prednisolone given at a tapering dose for five days along with fluids and multivitamin supplements. After seven days of oxytetracycline injection animal started intake of food. Tablet doxycycline @10mg/kg was given for 14 days. An uneventful clinical recovery was noticed post therapy.

DISCUSSION

Haemotropic mycoplasmas (hemoplasmas) have been reported in several mammalian species including dogs. This may cause severe haemolytic anaemia. But the investigations in dog had been hampered due to lack of adequate diagnostic techniques. This study expands the knowledge of diagnosis of canine mycoplasma by employing cytology and polymerase chain reaction assay in dogs. Severe regenerative anaemia and intermittent fever are the major clinical signs in case of haemotropic mycoplasma infection in dogs, clinical signs in the present study was in accordance with these findings. Canine Haemotropic mycoplasmas are cosmopolitan in distribution but in India very few cases has been reported. *Rhipicephalus sanguineus* is considered as major vector which is responsible for the transmission of the disease (Wengi *et al.,*2008) and these ticks are commonly encountered in this region. The anaemia in haemotropic mycoplasmosis is attributed to destruction of erythrocytes. The most common haematological abnormality was reported to be regenerative, macrocytic and normochromic anemia (Willi *et al.* 2005). Clinical signs in the present study are correlating with findings of Wengi *et al.*(2008), Saski *et al.*(2008).Cytological identification followed by a molecular level confirmation that is species specific Polymerase chain reaction was used as the diagnostic technique in the present study. Tetracyclines or fluroquinolones are the drug of choices and oral administration of doxycycline 5-10 mg/kg PO q12-24h for 14 to 21 days is also recommended for treating infections with haemotropic mycoplasma, Novacco *et al.* (2010). Prednisolone is indicated to control self-destruction of RBC in case of mycoplasmosis. Present case was treated successfully with inj. Oxytetracycline at the dose rate of 10mg/kg for five days along with Inj. Prednisolone (1 mg/kg) tapering dose.

CONCLUSION

A case of Haemotropic mycoplasmosis in a dog and its successful management is documented. The case was diagnosed based on cytology and PCR. The dog was

successfully treated with parenteral administration of oxytetracycline and supportive therapy with prednisolone and haematinics. Based on the present study it is recommended that Haemotropic mycoplasmosis should be included in the differential diagnosis of anaemia in immune compromised dogs.

Acknowledgements

The authors are extremely thankful to Dr. SeverineTasker lecturer in small animal medicine, the feline centre, School of Clinical Veterinary Science and Langford Veterinary Services, University of Bristol, Bristol, United Kingdom. For providing positive control of *Mycoplasma haemocanis*. Authors are grateful to The Dean, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University for providing necessary facilitiesfor the study.

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How to cite this article:

Tresamol P. V and Ameldev, P (2018) 'Molecular Confirmation and Therapeutic Management of Haemotropic Mycoplasmosis in A Dog, A Case Study', *International Journal of Current Advanced Research,* 07(7), pp. 13953-13955. DOI: http://dx.doi.org/10.24327/ijcar.2018.13955.2511

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