Diagnosis of feline haemoplasma infection using a real-time PCR assay

R G Lobetti[a*] and S Tasker[b]

ABSTRACT

Haemobartonella felis has been reclassified within the genus *Mycoplasma* as *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum', collectively referred to as the feline haemoplasmas. A total of 78 cats from the Johannesburg area that had blood samples submitted to a private veterinary laboratory were tested using a real-time polymerase chain reaction (PCR) assay able to detect and distinguish the two feline haemoplasma (basonym *Haemobartonella*) species. All samples had been diagnosed with haemoplasma infection by cytological examination of blood smears. Statistical analysis was performed to evaluate associations between haemoplasma status, age, and haematological and biochemical parameters. On PCR assay 43 cats (55 %) were haemoplasma negative, 25 (32.1 %) positive for '*Candidatus* Mycoplasma haemominutum', 5 (6.4 %) positive for *Mycoplasma haemofelis* and 5 (6.4 %) positive for both species. Significant inverse correlation was found between the amount of *M. haemofelis* DNA present in the blood and the haematocrit value. Cats that were positive for *M. haemofelis* showed macrocytic regenerative anaemia, monocytosis and thrombocytopaenia. This report documents the existence of both haemoplasma species in cats in South Africa.

Key words: cat, haematology, *Haemobartonella felis,* haemoplasma, PCR.

Lobetti R G, Tasker S **Diagnosis of feline haemoplasma infection using a real-time PCR assay**. *Journal of the South African Veterinary Association* (2004) 75(2): 94–99 (En.). Bryanston Veterinary Hospital, PO Box 67092, Bryanston, 2021 South Africa.

INTRODUCTION

Based on phylogenetic analysis of 16S rRNA gene sequences, *Haemobartonella felis* has been reclassified within the genus *Mycoplasma* as *Mycoplasma haemofelis*[13] and '*Candidatus* Mycoplasma haemominutum'[11]. Both species are collectively referred to as the feline haemoplasmas. Experimental infection with *M. haemofelis* often causes a severe haemolytic anaemia[2,7,22]. Conversely *'Candidatus* M. haemominutum' infection does not usually induce anaemia[7,22], although it has been suggested that co-infection with *'Candidatus* M. haemominutum' and feline retroviruses may result in anaemia[9].

The existence of feline haemoplasmas in South African cats was first reported in 1942[5]. No further studies have, however, been reported in the literature. Until recently, cytological examination of blood smears to visualise organisms on the surface of erythrocytes was relied upon to diagnose feline haemoplasma infection. Based on cytological diagnosis, feline haemoplasma infection has been recognised worldwide[1,3,6,10,12,14].

[a]Bryanston Veterinary Hospital, PO Box 67092, Bryanston, South Africa, 2021.

[b]Department of Clinical Veterinary Science, University of Bristol, Langford, Bristol, BS40 5DU, United Kingdom.

*Author for correspondence.
E-mail: rlobetti@mweb.co.za

Received: January 2004. Accepted: March 2004.

Recently, several studies have shown that conventional polymerase chain reaction (PCR) assays are more sensitive than cytology for the diagnosis of this infection[2,7,22]. In addition, PCR, unlike cytology, is able to definitively distinguish between *M. haemofelis* and *'Candidatus* M. haemominutum' infection. Recent prevalence studies using conventional PCR to detect feline haemoplasma infection have been carried out in the USA[11], UK[17] and Australia[21].

Real-time PCR allows the detection of amplicon accumulation as it is synthesised[4], so post-amplification steps, such as gel electrophoresis, is not required. The assay is therefore performed rapidly, and, since there is no need to open the reaction tubes following PCR, amplicon carry-over and false-positive results are far less likely than with conventional PCR. The amount of fluorescence generated in real-time PCR is proportional to the amount of accumulated PCR product, so measurement of fluorescence during the exponential phase of PCR provides an accurate means to quantify DNA template in an unknown sample. Quantification of DNA template may be of particu- lar importance with infectious agents such as the feline haemoplasmas, which do not invariably cause clinical disease in the host. A real-time PCR assay for the detection and quantification of feline haemoplasma DNA has recently been described[18].

The aim of this study was to evaluate the prevalence of infection with both *M. haemofelis* and *'Candidatus* M. haemominutum' in blood samples collected from cats in the Johannesburg area, using a real-time PCR assay, and to correlate the results with haematological and serum biochemistry parameters.

MATERIALS AND METHODS

Samples

Surplus EDTA-anticoagulated blood (minimum 100 $\mu\ell$) from 78 feline samples submitted to a private veterinary laboratory (Golden Veterinary Laboratories, Alberton, South Africa) for routine haematological testing was stored at –20 °C. The samples were obtained from cats with a variety of disease conditions and all had been diagnosed with haemoplasmosis on blood smear evaluation, stained with Cams Quick stain (CA Milsch, Krugersdorp, South Africa). The batched blood samples were then couriered to the Department of Veterinary Clinical Science, University of Bristol in the United Kingdom.

For each sample, the cat's age, haematological data, and any available biochemical data were recorded.

DNA extraction

Genomic DNA was prepared from 100 $\mu\ell$ of the EDTA whole blood samples using the DNeasy 96 Tissue Kit (Qiagen, Crawley, UK) according to the manufacturer's instructions. For each plate of DNA extractions performed, three 100 $\mu\ell$ aliquots of phosphate buffered saline underwent the DNA extraction protocol for subsequent PCR to screen for contamination during DNA extraction.

PCR amplification

All PCR amplifications were performed in 25 $\mu\ell$ reaction volumes. A feline haemoplasma real-time PCR assay[18] was performed on all samples. Briefly, this assay comprised feline haemoplasma-specific primers (Hf Forward 5'-ACGAAAGTCT GATGGAGCAATA-3' and Hf Reverse 5'-ACGCCCAATAAATCCGRATAAT-3')

(Life Technologies, Paisley, Scotland) and Taqman probes specific for either *M.haemofelis* (hexachloro-fluorescein [HEX]-TACGAGGGATAATTATGATAGTA CTTCGTGA-Black hole quencher [BHQ]1) or *'Candidatus* M. haemominutum' (6-carboxyfluorescein [FAM]-AGC TTGATAGGAAATGATTAAGCCTTGA-B HQ1) (Cruachem Ltd, Glasgow, Scotland). PCR reactions comprised 12.5 $\mu\ell$ 2X Platinum Quantitative PCR Supermix-UDG (Invitrogen Ltd, Glasgow, UK), 260 nM of each primer, 300 nM FAM Taqman probe, 200 nM HEX Taqman probe, 6 mM $MgCl_2$ final concentration, 1 U additional Platinum *Taq* polymerase (2.2U per 25 $\mu\ell$ reaction), and 2 $\mu\ell$ of template DNA, made up to a final volume of 25 $\mu\ell$ with water. PCR was performed using an iCycler IQ system (Bio-Rad Laboratories Ltd, London) with an initial incubation at 50 °C for 3 minutes, then 95 °C for 2 minutes followed by 45 cycles of 95 °C for 5 seconds and 58 °C for 30 seconds. Fluorescence was detected at 530 nm and 570 nm at each annealing step. All samples were run in triplicate. DNA samples from known infected and non-infected cats and water were subjected to PCR as positive and negative controls, respectively. To confirm the presence of amplifiable DNA in the samples, and for standardisation across the samples, a real-time PCR for the detection of feline 28S rDNA was performed on all specimens. This assay comprised 12.5 $\mu\ell$ 2X Qiagen Hotstart Enzyme (Qiagen), 200 nM of each primer (28S rDNA forward 5'-AGCAGGAGG TGTTGGAAGAG-3' and 28S rDNA reverse 5'-AGGGAGAGCCTAAATCAA AGG-3'), 100 nM 28S rDNA Taqman probe (TEXAS RED-TGGCTTGTGGCA GCCAAGTGT-BHQ2), 4.5 mM $MgCl_2$ final concentration and 2 $\mu\ell$ of template DNA, made up to a final volume of 25 $\mu\ell$ with water. PCR was performed using an iCycler IQ system with an initial incubation at 95 °C for 15 minutes followed by 45 cycles of 95 °C for 10 seconds and 60 °C for 15 seconds. Fluorescence was detected at 620 nm at each annealing step.

Statistical analysis

Descriptive statistics were obtained for age, haematological parameters (red cell count (RBC), haemoglobin (Hb), haematocrit (Ht), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), total white cell count (WCC), neutrophil count, band neutrophil count, monocyte count, lymphocyte count, eosinophil count, basophil count, and platelet count, serum biochemistry (total serum protein, albumin, globulin, alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), urea, and creatinine) and 28S rDNA real-time threshold cycle (C_T) values. Based on the results of the real-time haemoplasma PCR assay, cats were divided into four groups: negative, '*Candidatus* M. haemominutum'-only positive, *M. haemofelis*-only positive, and dual positive (co-infected cats). These four groups were statistically analysed for differences in age, 28S rDNA C_T values, haematological or biochemical variables.

Normally distributed data were analysed using analysis of variance (ANOVA) and non-normally distributed data were analysed using Kruskal-Wallis ANOVA. Statistical evaluation was carried out using SPSS for Windows 10.1.0 (SPSS Inc., Chicago, Illinois). Descriptive statistics were obtained for each variable and normality was tested for using the Kolmogorov-Smirnov test. Logarithmic transformation of some variables with a skewed distribution resulted in normal distributions for parametric analysis. These variables were WCC, band neutrophil, reticulocyte and platelet counts, ALT, SAP, urea, and creatinine concentrations. Square root transformation of the eosinophil counts resulted in a normal distribution. Logarithmic and square root transformation of both lymphocyte and basophil counts did not result in normal distributions, necessitating non-parametric analysis. Although normally distributed, age, Hb and Ht values showed a lack of homogeneity of variances across the four groups for one-way ANOVA analysis, necessitating the use of Kruskal-Wallis ANOVA for these data. When a statistically significant difference was identified between the groups, the nature of the difference between individual groups was determined using the Tukey *post hoc* multiple comparisons test for normally distributed data and the Mann-Whitney *U*-test for non-normally distributed data. A significant association was taken to be one with a P value ≤0.05. Bivariate correlation between haemoplasma DNA and Ht was measured by determination of Spearman's correlation coefficients.

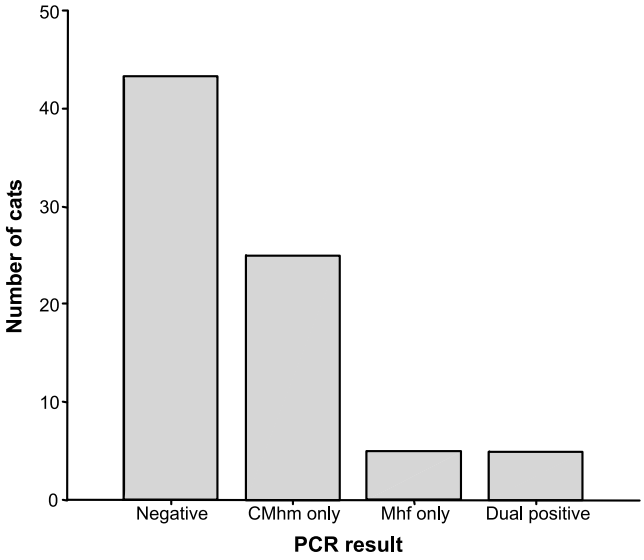

Fig. 1: **Detection of haemoplasmas by real-time PCR in 78 cat samples. CMhm = *'Candidatus* M. haemominutum'; Mhf = *Mycoplasma haemofelis.***

RESULTS

Haemoplasma real-time PCR

Seventy-eight blood samples underwent real-time PCR. Twenty-five cats (32.1 %) were PCR positive for *'Candidatus* M. haemominutum' alone, 5 (6.4 %) were positive for *M. haemofelis* alone, and 5 (6.4 %) were dual positive (co-infected) for both species (Fig. 1). Overall, of the 78 samples, 35 (44.9 %) were positive for one or both feline haemoplasma species. All positive and negative controls in the PCR assays were positive and negative, respectively.

Feline 28S rDNA real-time PCR

All samples submitted for haemoplasma real-time PCR gave positive results with the feline 28S rDNA assay, indicating the presence of amplifiable DNA. The C_T values ranged from 22.4 to 41.3 (mean 27.4, median 26.6, standard deviation 3.6, standard error 0.41). The sample with a C_T value of 41.3 generated a positive result with the haemoplasma real-time PCR assay for *'Candidatus* M. haemominutum'. All water controls gave negative results. No significant differences in 28S rDNA C_T values were found between the four groups based on haemoplasma PCR results.

Characteristics of cases

Descriptive statistics for continuous

Table 1: **Descriptive statistics for haematological and serum biochemistry variables in cats with natural haemoplasma infection.**

Parameter	Number	Minimum	Maximum	Median	Mean	SD	SE
Age (years)	64	1.0	19.0	6.0	7.5	5.11	0.64
Haematocrit (l/ℓ)	78	8.0	47.0	29.0	27.75	8.77	0.99
Haemoglobin (g/ℓ)	78	26	163	105	97	32	3.6
Red cell count ($\times10^{12}$/ℓ)	78	1.44	11.70	6.00	6.50	2.39	0.27
Mean cell volume (fl)	78	30	72	43	44.5	7.39	0.84
Mean cell haemoglobin concentration (g/dℓ)	78	10	45	35	34.7	3.69	0.42
White cell count ($\times10^9$/ℓ)	78	0.13	48.30	7.86	6.31[a]	2.75[a]	1.12[a]
Platelets ($\times10^9$/ℓ)	78	9	844	155.5	118.0[a]	3.09[a]	1.14[a]
Neutrophils ($\times10^9$/ℓ)	70	0.4	20.7	5.13	6.25	4.38	0.52
Band neutrophils ($\times10^9$/l)	70	0	1.89	0.33	0.42[a]	0.34[a]	0.04[ac]
Lymphocytes ($\times10^9$/ℓ)	70	0	44	0.72	1.80	5.32	0.636
Monocytes ($\times10^9$/ℓ)	70	0	1	0.39	0.47	0.351	0.042
Eosinophils ($\times10^9$/ℓ)	70	0	3.68	0.11	1.06[b]	0.18[b]	0.003[bc]
Basophils ($\times10^9$/ℓ)	70	0	0.28	0	0.04	0.061	0.007
Reticulocytes %	75	0	20	2	1.93[a]	1.04[a]	0.09[a]
Total serum protein	62	45	98	70	70.44	10.48	1.33
Albumin	61	12	47	27	27.44	6.15	0.79
Globulin	61	29	74	40	43.15	9.96	1.28
ALT	59	5	1033	74	87.97[a]	2.77[a]	1.14[a]
ALP	54	10	317	33	35.49[a]	2.27[a]	1.12[a]
Urea	58	1.9	61.5	7.7	8.93[a]	2.07[a]	1.10[a]
Creatinine	59	32	949	113	131.1[a]	2.03[a]	1.10[a]

[a]Geometric mean, standard deviation (SD) and standard error (SE).
[b]Square root transformed mean, standard deviation (SD) and standard error (SE).
[c]Variables with non-normally distributed data.

variables, including age, haematological and biochemical parameters are shown in Table 1.

Age

No significant difference in age was found between the four groups based on haemoplasma PCR results (Fig. 2).

Haematological parameters

Of the haematological variables evaluated by ANOVA or Kruskal-Wallis ANOVA, six were significantly different between the four groups based on haemoplasma PCR results. These were RBC ($P < 0.001$), Hb ($P = 0.001$), Ht ($P = 0.001$), MCV ($P < 0.001$), monocyte count ($P = 0.04$) and platelet count ($P = 0.02$) (Fig. 3).

The RBC counts of the *M. haemofelis*-only group were significantly lower than both the *'Candidatus* M. haemominutum'-only group ($P = 0.001$) and the negative group ($P < 0.001$) while the dual-positive group RBC counts were significantly lower than those of the negative group only ($P = 0.02$). There was no significant difference in the RBC counts of the negative group and the *'Candidatus* M. haemominutum'-only group ($P = 0.36$).

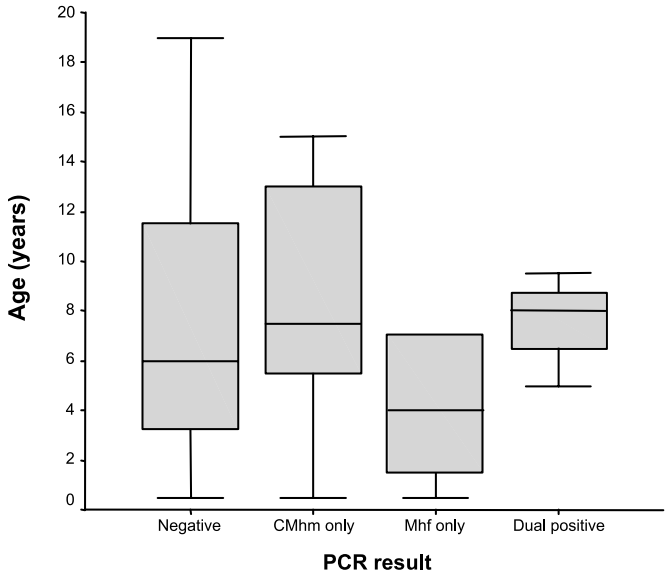

Fig. 2: **Age distribution of 78 cats with natural haemoplasma infection grouped by haemoplasma PCR result. CMhm = *'Candidatus* M. haemominutum'; Mhf: *Mycoplasma haemofelis.* Boxes represent the 25th, 50th** (median) **and 75th quartiles with whiskers extending to the greatest and smallest values.**

The Hb values of the *M. haemofelis*-only group were significantly lower than those of all the other groups; the dual-positive group ($P = 0.009$), the *'Candidatus* M. haemominutum'-only group ($P = 0.002$) and the negative group ($P < 0.001$). The Hb values of the dual-positive group were also significantly lower than those of the negative group ($P = 0.02$) but significance was not reached with the *'Candidatus M.* haemominutum'-only group ($P = 0.09$). There was no significant difference in the Hb values of the negative group and the *'Candidatus* M. haemominutum'-only group ($P = 0.41$).

The results of statistical analysis of Ht values were similar to those for Hb values. The Ht values of the *M. haemofelis*-only group were significantly lower than those of all the other groups; the dual-positive group ($P = 0.01$), the *'Candidatus* M. haemominutum'-only group ($P = 0.003$) and the negative group ($P < 0.001$). The Ht values of the dual-positive group were also significantly lower than those of the negative group ($P = 0.02$) but significance was not reached with the *'Candidatus* M. haemominutum'-only group ($P = 0.09$). There was no significant difference in the Ht values of the negative group and the *'Candidatus* M. haemominutum'-only group ($P = 0.449$).

Bivariate analysis revealed a significant correlation between the Ht and DNA from *M. haemofelis*-positive cats (Spearman $r = 0.82$, $P = 0.023$) but no significant

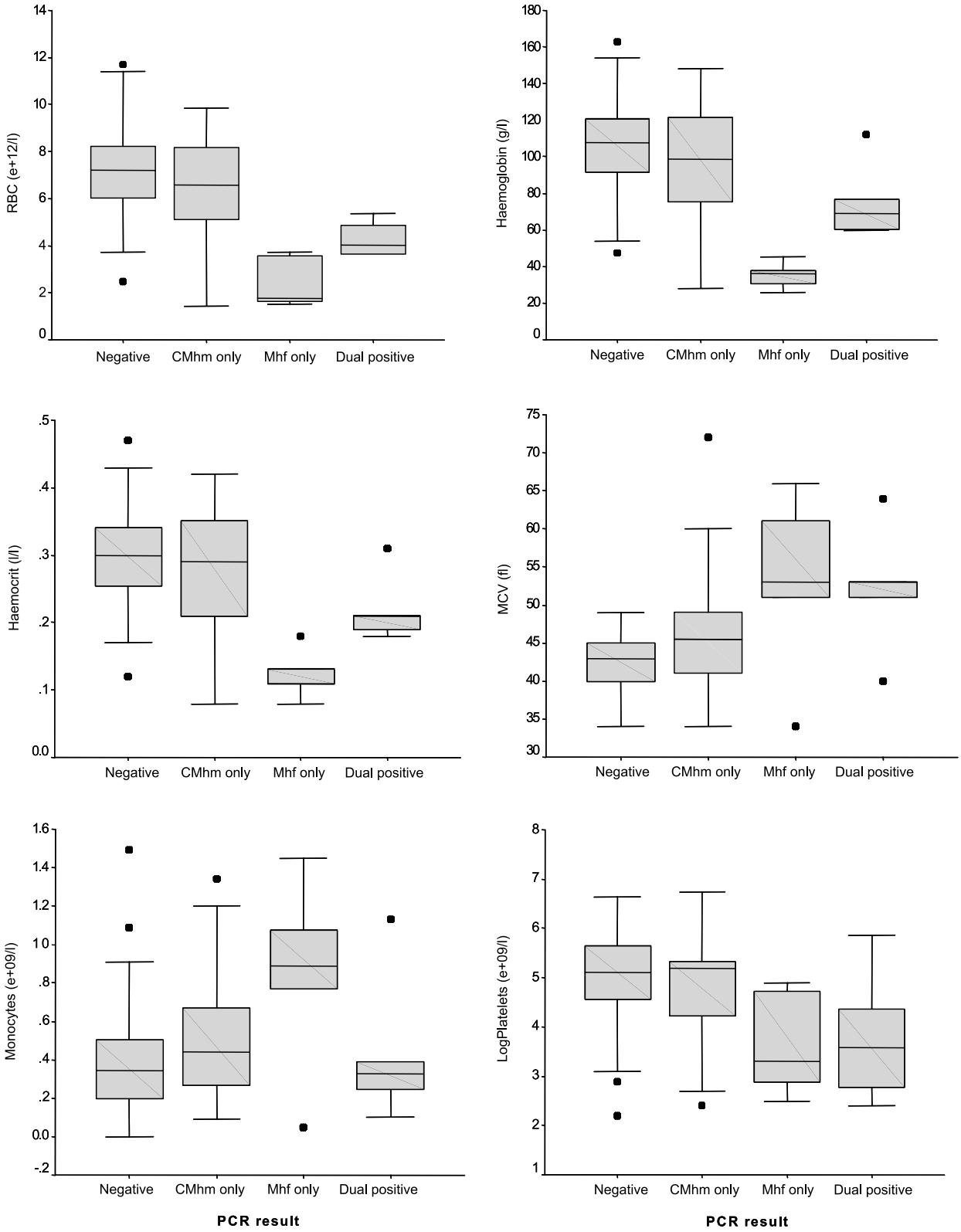

Fig. 3: **Distribution of selected haematological variables in cats with natural haemoplasma infection grouped by haemoplasma PCR result. CMhm = *'Candidatus M. haemominutum'*; Mhf = *Mycoplasma haemofelis*. Boxes represent the 25th, 50th** (median) **and 75th quartiles with whiskers extending to the greatest and smallest values. Black dots indicate outliers** (cases with values greater than 1.5 box lengths from the upper or lower edge of the box).

correlation was evident between the Ht and DNA from cats positive for '*Candidatus* M. haemominutum' (Spearman r = 0.14, P = 0.448).

The MCV values of both the *M. haemofelis*-only and the dual-positive groups were significantly greater than those of the negative group (P = 0.005 and P = 0.01, respectively). No other significant differences in MCV were found between the four groups.

The monocyte count of the *M. haemofelis*-only group was significantly greater (P = 0.03) than that of the negative group. No other significant differences in monocyte counts were found between the four groups.

The *M. haemofelis*-only group had lower platelet counts than the negative group (P = 0.05). No other significant differences were found between the groups.

Serum biochemical parameters

None of the biochemical variables evaluated showed any significant differences between the four groups based on haemoplasma PCR results.

DISCUSSION

This is the first study in South African cats to report the prevalence of both *'Candidatus* M. haemominutum' and *M. haemofelis* in a convenience-sample of feline patients. The successful amplification of haemoplasma DNA in these specimens confirms, for the first time, the existence of both *'Candidatus M. haemominutum'* and *M. haemofelis* in South Africa. Indeed, sequencing of the full length of the 16S rRNA gene of a number of South African haemoplasma isolates has confirmed the existence of species with near identical 16S rRNA sequences to those previously reported in the UK, USA and Australia[16,20].

There is currently no recognised accepted gold standard definitive diagnostic method for the diagnosis of haemoplasma infections. PCR has been shown to be more sensitive than cytology for diagnosis in several studies[8,11,22] with positive PCR results obtained with samples negative on cytology, including experimentally infected cats. To the best of our knowledge the primers used in the assays of the current studies are known to be specific for haemoplasma species. They do not amplify template DNA from other bacterial organisms associated with bacteraemia in cats[11] and sequence data from different worldwide isolates[20] show that these primers anneal to regions of the 16S rRNA gene, which are conserved in all the isolates examined. The fluorogenic probes used in the real-time PCR assay enable distinction between *M. haemofelis* and *'Candidatus* M. haemominutum'. Although morphological differences between the species have been described[7], examination of blood smears cannot reliably distinguish between the species[22]. Additionally, cytology is based on subjective interpretation of erythrocyte-associated bodies. False positive cytology diagnoses have been reported when feline erythrocytic inclusions such as Howell-Jolly bodies or stain precipitate are mistaken for haemoplasma organisms[3,4].

The present study was carried out using a convenience-sampled population, the limitations of which have been discussed previously[15]. When assessing prevalence of infection, truly random samples should be used to generate data, but such samples are difficult to obtain in companion animal studies. Without doubt, differences exist in the sex, age and breed distributions of the cats sampled compared with the general South African cat population. The cats studied also differ from cats in the general population because the majority were being investigated by veterinarians.

In previous studies[11, 17,21] the prevalence of *'Candidatus* M. haemominutum' infection was 23.1 % cats in Australia, 12.7 % in the USA and 16.9 % in the UK. *M. haemofelis* infection was less common (4.1 % cats in Australia, 4.5 % in the USA and 1.4 % in the UK) and dual infection was rare (0.7 % of cats in Australia, 2.3 % in the USA and 0.2 % in the UK). In this study *'Candidatus* M. haemominutum' infection was 32.1 %, *M. haemofelis* infection was 6.4 %, and dual infection was 6.4 %. Direct comparisons of the prevalence reported in these different studies are of limited value because of likely differences in the populations sampled. Indeed, the USA and Australian studies as well as the current study were performed on samples received after requests for blood samples from suspected haemoplasma-infected cats were made to local veterinarians. In the USA study, 37 % of cats evaluated for haemoplasma infection by PCR were suspected of harbouring haemoplasmas based on the presence of anaemia, fever and/or cytological evidence of infection. This study included all cats that had been diagnosed with feline haemoplasma infection based on cytology. Limitations therefore exist with extrapolation of results from this study to the general cat population in South Africa.

The clinical significance of a positive PCR result for *'Candidatus* M. haemominutum' is not known. Studies have shown a relatively high prevalence in cats, both in healthy and sick groups, confirming that infection with this organism is quite common in different parts of the world[17,20,21]. A positive PCR result for *'Candidatus* M. haemominutum' does not indicate that there is disease associated with infection. In studies in experimentally infected cats, mild or no pathogenicity associated with this species has been documented[7,9,22], and in naturally infected cats there was no significant difference in prevalence of infection with *'Candidatus* M. haemominutum' between anaemic and non-anaemic cats[11]. None of the haematological variables measured in either this study or in the UK study showed significant statistical differences between *'Candidatus* M. haemominutum'-infected cats and negative cats.

In the present study, both *M. haemofelis*-infected cats and those co-infected with both haemoplasma species had significantly lower RBC counts, Hb and Ht values, and significantly higher MCV values, compared with negative cats. Thus, an association between *M. haemofelis* and a regenerative anaemic status could be hypothesised from these results. It has been suggested in one experimental study[22] that dual infection with both haemoplasma species may result in more severe disease than infection with *M. haemofelis* only, since co-infected cats showed more pronounced clinical abnormalities in terms of severity and duration of anaemia, variations in body temperature and leucocyte abnormalities although they did not show more severe haematological changes than those infected with *M. haemofelis* alone. Indeed, the *M. haemofelis*-alone-infected group had significantly lower Hb and Ht values than the co-infected group. No significant association between *M. haemofelis* and anaemia was found in the UK study[17] whereas an association was found in the Australian study[21]. Such differences could simply be a reflection of sample numbers in the studies or varying pathogencity between different *M. haemofelis* isolates from different parts of the world. Since some healthy, non-anaemic cats were found to be *M. haemofelis* infected in the present study, a positive PCR result for *M. haemofelis,* as for '*Candidatus* M. haemominutum', does not indicate that there is disease associated with infection.

The relatively low number of cats infected with *M. haemofelis* in these studies limits the reliable determination of associations with infection. In the UK study[17], there was no significant difference in age between the *M. haemofelis*-infected and the negative groups, although a significant difference was seen between the *M. haemofelis* and the *'Candidatus* M. haemominutum'-infected cats, due to the latter being older. In the UK study, a significant association between FIV status and *M. haemofelis* infection was found, but as described for '*Candidatus* M. haemominutum', other factors such as breed may be confounding effects. Further studies using larger numbers of *M. haemofelis*-infected cats will be required to characterise true associations.

Positive PCR results (for both haemoplasma species) have been reported in asymptomatic cats[17], therefore the significance of a positive PCR result should always be interpreted in the light of observed physical findings and haematological features of the patient[19]. Real-time PCR assays offer quantitative information on the amount of haemoplasma DNA present[18] in addition to confirming the presence of haemoplasma DNA. In this study it was hypothesised that those cases in which haemoplasma infection was thought to be the cause of the anaemia

should have the greatest quantities of haemoplasma DNA present in blood. The correlation data enabled evaluation of the relationship between Ht values and the quantity of haemoplasma DNA present in blood samples. Significant positive correlation was seen with *M. haemofelis*-infected cats but not with the '*Candidatus* M. haemominutum'-infected cats. This is not surprising given the differences in pathogenicity reported for the two species. The significant positive correlation reported in the *M. haemofelis*-infected cats occurred despite the inclusion of only a very small number of cats.

Future studies should further evaluate the use of quantified haemoplasma DNA data from a larger and more representative cohort of cats since this may help with the interpretation of the significance of a positive PCR result, particularly with *M. haemofelis.* In particular, quantified data may help distinguish between chronic asymptomatic haemoplasma carrier cats and those with active infection.

ACKNOWLEDGEMENTS

The authors would like to thank Golden Veterinary Laboratories for providing the samples. Séverine Tasker was partly funded by the Royal College of Veterinary Surgeons Trust Fund for Feline Research.

REFERENCES

1. Anderson D C, Charleston W A G 1967 *Haemobartonella felis. New Zealand Veterinary Journal* 15: 47
2. Berent L M, Messick J B, Cooper, S K 1998 Detection of *Haemobartonella felis* in cats with experimentally induced acute and chronic infections, using a polymerase chain reaction assay. *American Journal of Veterinary Research* 59: 1215–1220
3. Bobade P A, Akinyemi J O 1981 A case of haemobartonellosis in a cat in Ibadan. *Nigerian Veterinary Journal* 10: 23–25
4. Bustin S A 2000 Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology* 25: 169–193
5. Clark R 1942 *Eperythrozoon felis* (sp. nov.) in a cat. *Journal of the South African Veterinary Medical Association* 13: 15–16
6. Flagstad A, Larsen S A 1969 The occurrence of feline infectious anaemia in Denmark. *Nordisk Veterinaer Medicin* 21: 129–141
7. Foley J E, Harrus S, Poland A, Chomel B, Pedersen N C 1998 Molecular, clinical, and pathologic comparison of two distinct strains of *Haemobartonella felis* in domestic cats. *American Journal of Veterinary Research* 59: 1581–1588
8. Foley J E, Pedersen N C 2001 '*Candidatus* Mycoplasma haemominutum', a low-virulence epierythrocytic parasite of cats. *International Journal of Systematic and Evolutionary Microbiology* 51: 815–817
9. George J W, Rideout B A, Griffey, S M, Pedersen N C 2002 Effect of pre-existing FeLV infection or FeLV and feline immunodeficiency virus co-infection on pathogenicity of the small variant of *Haemobartonella felis* in cats. *American Journal of Veterinary Research* 63: 1172–1178
10. Hatakka M 1972 Haemobartonellosis in the domestic cat. *Acta Veterinaria Scandinavica* 13: 323–331
11. Jensen W A, Lappin, M R, Kamkar S, Reagen W J 2001 Use of a polymerase chain reaction assay to detect and differentiate two strains of *Haemobartonella felis* infection in naturally infected cats. *American Journal of Veterinary Research* 62: 604–608
12. Maede Y, Hata R, Shibata H, Niiyama M, Too K, Sonoda M 1974 Clinical observation on six cases of feline infectious anaemia. *Journal of the Japanese Veterinary Medical Association* 27: 267–272
13. Neimark H, Johansson K E, Rikihisa Y, Tully J G 2001 Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of '*Candidatus* Mycoplasma haemofelis', '*Candidatus* Mycoplasma haemomuris, *Candidatus* Mycoplasma haemosuis' and *Candidatus* Mycoplasma wenyonii'. *International Journal of Systematic and Evolutionary Microbiology* 51: 891–899
14. Seamer J, Douglas S W 1959 A new blood parasite of British cats. *The Veterinary Record* 71: 405–408
15. Sukura A, Grohn Y T, Junttila J, Palolahti T 1992 Association between feline immunodeficiency virus antibodies and host characteristics in Finnish cats. *Acta Veterinaria Scandinavica* 33: 325–334
16. Tasker S 2002 *Feline haemoplasmas – detection, infection, dynamics and distribution.* PhD thesis, University of Bristol, Bristol, United Kingdom
17. Tasker S, Binns S H, Day M J, Gruffydd-Jones T J, Harbour D A, Helps C R, Jensen W A, Olver C S, Lappin M R 2003 Use of a PCR assay to assess prevalence and risk factors for *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' in cats in the United Kingdom. *The Veterinary Record* 152: 193–198
18. Tasker S, Helps C R, Day M J, Gruffydd-Jones T J, Harbour D A 2003 Use of real-time PCR to detect and quantify *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' DNA. *Journal of Clinical Microbiology* 41: 439–441
19. Tasker S, Lappin M R 2002 *Haemobartonella felis:* recent developments in diagnosis and treatment. *Journal of Feline Medicine and Surgery* 4: 3–11
20. Tasker S, Helps C R, Day M J, Harbour D A, Shaw S E, Harrus S, Baneth G, Lobetti R G, Malik R, Beaufils J, Belford CR, Gruffydd-Jones T J 2003 Phylogenetic analysis of Haemoplasma species – an international study. *Journal of Clinical Microbiology* 41: 3877–3880
21. Tasker S, Braddock J A, Baral R, Helps C R, Day M J, Gruffydd-Jones T J, Malik R In press Diagnosis of feline haemoplasma infection in Australian cats using a real-time PCR assay. *Journal of Feline Medicine and Surgery*
22. Westfall D S, Jensen W A, Reagan W J, Radecki S V, Lappin M R 2001 Inoculation of two genotypes of *Haemobartonella felis* (California and Ohio variants) to induce infection in cats and the response to treatment with azithromycin. *American Journal of Veterinary Research* 62: 687–681