

Feasibility of using point-of-care c-reactive protein assays in the diagnosis of canine lower respiratory tract infections- a basis for targeted antibiotic use?



**University of
Nottingham**
UK | CHINA | MALAYSIA

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CVS Laboratories/University of Nottingham Combined Residency and
MVM Programme in Veterinary Clinical Pathology

Thesis submitted for Master of Veterinary Medicine (MVM)
University of Nottingham

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Abbreviations

α 1-AGP – Alpha-1 acid glycoprotein
ACVP – American College of Veterinary Pathology
APP – Acute phase protein
ASVCP – American Society of Veterinary Clinical Pathology
BAL – Bronchoalveolar lavage
BC – Beckman Coulter
BSAVA – British Small Animal Veterinary Association
Cp – Ceruloplasmin
CRP – C-reactive protein
CV – Coefficient of variation
ECVCP – European College of Veterinary Clinical Pathology
ELISA – Enzyme-linked immunosorbent assay
Fb – Fibrinogen
Hp – Haptoglobin
IL – Interleukin
ISO – International Organization for Standardization
IT – Immunoturbidometric
LoQ – Limit of Quantification
NAD – No abnormalities detected
NICE – The National Institute for Health and Care Excellence
RCPath – Royal College of Pathologists
SAA – Serum Amyloid A
SIRS – Systemic inflammatory response syndrome
SVMS – School of Veterinary Medicine and Science
TE – Total error
TE_a – Total allowable error
TE_{obs} – Total observed error
TNF – Tumour necrosis factor
TTW – Transtracheal wash
TW – Tracheal wash
VIN – Veterinary Information Network

Chapter 1

Clinical Training and Exam Preparation

1. Introduction

This scholarship was a combined clinical residency and Masters degree in Veterinary Clinical Pathology, and was created as a shared industry and university-based programme, partnered between the University of Nottingham and CVS Laboratories (CVS Group plc, Diss Norfolk UK), particularly Finn Pathologists in Norfolk. The programme started in October 2017, firstly based at the School of Veterinary Medicine and Science, Nottingham with occasional visits to Finn Pathologists. I continued to be based at Nottingham until February 2019, after which I switched to being based at Finn full time with occasional visits back to Nottingham for the remainder of the programme. The overall goal of the clinical training is to sit the Royal College of Pathologists Fellowship examinations (FRCPath), with further potential to sit the American College of Veterinary Pathologists (ACVP) examinations thereafter, if desired. Throughout the residency I have been supervised by Peter Graham (ECVCP diplomate), Rachel Dean, Jenny Stavisky and Steve Dunham at Nottingham; and Rand Wilson (ACVP diplomate) as my external supervisor at Finn Pathologists, along with other pathologists (each with ACVP/ECVCP/FRCPath certification) at Finn Pathologists. Training in clinical pathology is divided into five core areas: general pathology, haematology, biochemistry, cytology, and laboratory quality management. Further supplementary topics such as endocrinology, infectious diseases and immunology, and general internal medicine are also integrated to a lesser degree. Cases were seen from most common domestic species, mainly cats and dogs, with smaller numbers of cases from horses, farm animals, small mammals and exotic animals.

In addition to the clinical training, which is required to be at least 75% of the time during the course, I have undertaken a research project in the field of clinical pathology, which is detailed in further sections.

The following sections describe my clinical and professional development and other activities completed during the clinical training scholarship.

1.1. RCPATH Veterinary Clinical Pathology Specialty Training Programme

Objectives and Competencies

Three categories of competencies and outcomes are detailed as objectives for the programme by the Royal College of Pathologists, with 22 listed specific outcomes across these categories:

A. Outcomes and competencies as a scholar and scientist:

1. Understand the pathophysiologic bases for and aetiopathogenesis of disease, with an understanding of comparative pathology across a variety of species.
2. Understand the One Health concept and importance of inter-relationships between human and animal disease, and the importance of the veterinary clinical pathologist in recognition of emerging diseases.
3. Appreciate the importance of clinical pathology in determination of health and disease, disease diagnosis, determination of prognosis, monitoring of health and disease and monitoring of response to treatment(s).
4. Appreciate the unique position of pathology and clinical pathology as the interface between clinical medicine and the basic sciences.
5. Ability to critically appraise the literature and other sources of information (including people, presentations, books, journals, the internet or other sources) in order to distinguish between reputable and reliable sources of information and those that should be disregarded or upon which less emphasis should be placed. Should be able to critically evaluate journal articles and presentations and other sources of information with regard to the techniques, analyses, interpretation and conclusions.

6. Ability to organise, synthesise and translate information to and from the 'language of the laboratory' to communicate it to and with a variety of audiences, including other veterinarians at a variety of levels and positions in practice, veterinary nurses, laboratory technicians, laboratory managers, owners and other lay persons as stakeholders in the practice of veterinary medicine.

B. Outcomes and competencies as a laboratorian/pathologist/veterinary consultant

7. Appreciate the role of the clinical pathologist as an interpreter of meaning, educator, and consultant for laboratory testing and interpretation of laboratory results.
8. Appreciate the role of the clinical pathologist as the link between the clinician and the laboratory, and the sometimes conflicting role as an advocate for the laboratory, the patient and the clinician.
9. Understand methods and principles underlying modern laboratory analyses of various types, with the ability to help troubleshoot technical and/or quality- related issues associated with the instruments within the laboratory and apply appropriate quality assurance and quality control.
10. Ability to communicate well and interact appropriately with others - in writing, verbally, (in person, including presentations or teaching), and on the telephone. This includes working as part of a team and in showing initiative and ability to work independently, as needed.
11. Ability to make accurate interpretations of laboratory data (from various areas and across various areas in order to tie together a variety of laboratory data) and provide useful information to laboratory clients. This should include basic analytical thought processes and synthetic abilities and the ability to provide differential diagnoses, where appropriate, with communication regarding the order of most likely probability.
12. Understand the principles of quality assurance and quality control, with ability to apply these in the various departments and ability to help troubleshoot problems, implement solutions and monitor results of ongoing quality improvements. This should include the processes of quality planning, quality assessment, quality control and continuous quality improvement.
13. Understand and apply basic statistical techniques useful in the laboratory, most often in relation to QA and QC method/instrument validation but also in basic research using laboratory data.
14. Understand the processes of and importance of assay, instrument and method validation appropriate for different types of testing within the laboratory.
15. Understand issues regarding Information Technology (IT) and Laboratory Information Systems (LIMS) - including security, backup and basic functions.
16. Understand general health and safety regulations and specifically those items of importance in the veterinary laboratory. This includes a good working knowledge of zoonotic diseases (those that may pass from animals to humans) and reverse

zoonotic diseases (those that may pass from humans to animals) and those that may occur in both humans and animals due to shared environment and opportunities for exposure to pathogens.

C. Outcomes and competencies as a veterinary medical professional

17. Ability to recognise and work within personal competencies and to seek additional help, advice or guidance, when and as needed.
18. Able to organise workload and demonstrate good time management skills in the workplace, including service, teaching and/or research, or other job tasks. This should include the ability to work as part of a team, as well as taking initiative and personal responsibility when working independently.
19. Understand personal strengths and weaknesses and personality type, and how this affects goal-setting, approach to the profession, personal and professional conduct, with a view to providing a good work-personal life balance and good mental health throughout a working life.
20. Understand the ethics and values expected in the practice of veterinary clinical pathology and veterinary medicine, in general.
21. Be able to use a variety of methods and techniques to undertake ongoing study for board examination as part of the training program, ongoing continuing professional development and a commitment to life-long learning.
22. Ability to identify ongoing continuing professional development needs, make good choices in choosing continuing educational opportunities to meet these needs, and to reflect upon their importance and effect on behaviour and practices.

1.2. General Training and Clinical Duties - University of Nottingham

The focus in the first year and early part of the second year whilst at the University of Nottingham was on the general pathology, laboratory quality management and biochemistry sections of training, along with some basic haematology and cytology preparation. Although cytology and haematology training were not the focus, batches of cases were regularly provided for review from our external partner laboratories within CVS – Axiom, Greendale, and Finn Pathologists. Cases were provided with submission forms and a capsule history. Access to the lab databases to gain further information on the cases was

possible if needed, allowing review of the completed case once reported. The cases were then also subsequently discussed and reviewed with Peter Graham using a multi-head microscope.

I was also involved with the provision of the clinical pathology service to Pride Veterinary Hospital in Derby two days per week. Cytology and haematology cases were submitted from the first opinion general practice and Blue Cross cases, and occasionally referral cases from specialist clinicians on-site. In addition, as part of my training in laboratory quality management, I was involved in a project at the clinical pathology laboratory of Pride veterinary hospital to assess the performance of the biochemistry analyser and decide on appropriate quality control rules to monitor the assays using Sigma metrics. This was a useful opportunity to implement these methods in a clinical setting and observe first-hand the potential benefit of quantifying the quality of the performance of the tests used in lab, to enable selection of the most efficient quality control procedures.

1.3. General Training and Clinical Duties - Finn Pathologists

As part of the joint industry and university residency programme I moved to Finn Pathologists to complete the second half of my training in February 2019. This section of the training enabled me to experience a relatively large commercial diagnostic laboratory and to increase my cytology and haematology caseload under the supervision of further board-certified clinical pathologists.

The laboratory received between 80-130 cytology submissions per day from first opinion and referral practices from throughout the UK and various international sites. I would read between 10 and 30 of these cases each day I was involved in diagnostic case work. Cases were predominantly from cats and dogs, however occasional large animal and exotic animal cases were also received. Unfortunately, the number of large animal and exotic cases completed so far are lower than expected due to two planned externship placements to a laboratory which has a higher proportion of these types of cases being cancelled as a result of the COVID-19 pandemic. A planned externship placement to a pharmaceutical company to gain experience in toxicologic clinical pathology was also cancelled.

After I had examined the cytology slides and written a report, the cases were subsequently reviewed by the supervising pathologist who advised on any corrections that were needed. Approximately 8-20 haematology cases were designated for review by a clinical pathologist each day. These cases could be identified for this further review by the haematology technician who originally assessed the case or the review may be ordered directly by the referring clinician. The haematology data, blood smears, and any supplementary test results were then reviewed, and a written report and interpretation of the case would be provided. Occasional bone marrow histopathology cases were also received, which were reviewed by the clinical pathology team.

On commencing my third year of training, I started reporting cases without supervision, with any assistance available from the on-site pathologists if requested.

Examples of cytology, haematology and bone marrow histopathology reports are presented below.

For one afternoon per week I also reviewed and provided comments for biochemistry, endocrinology and infectious disease cases, with assistance from the internal medicine consultant team if required. The farm animal team provided occasional farm animal biochemistry cases for me to review. Whilst at Finn Pathologists, one day per week was also allocated to study time, research and meetings/journal club. I have also had the opportunity to be involved in the histopathology service at Finn with the anatomic pathologists, spending two weeks viewing the process that biopsies follow from sample trimming and processing, through to interpretation and report writing by the pathologist, and being involved in reporting cases under supervision. Additional tutorial/CPD seminars were also provided on a range of topics related to histopathology by various pathologists.

Since being situated away from the university from February 2019 I attended regular video meetings with Peter Graham and the anatomic pathology team at Nottingham to continue with biochemistry and general pathology training and discuss the masters project.

Example cytology report:

20200819055311

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E-mail: email@finnpathologists.com

START OF REPORT

Patient:		
Access Number:		
ID:		
ASG:	12 Years 1 Month Dog (Canine - Domestic) (Staffordshire Bull Terrier) MaleNeutered	
Report Date:		
Date Sample Received:		

Test Results

(CYTO4-6) Cytology up to 12 slides from up to 6 sites
Cytology Report

SAMPLE RECEIVED:

12x FNA slides submitted from liver.

History of vomiting and diarrhoea, weight loss, PU/PD and increased appetite despite poor body condition. Increased liver enzyme activities with a 4 x 4cm hyperechoic focal liver mass in the left caudal lobe. Overall enlarge hyperechoic liver with thickened hyperechoic gall bladder wall.

MICROSCOPIC DESCRIPTION:

The slides are moderate to highly cellular with generally adequate cellular preservation against a moderately haemodiluted background. There are large numbers of polygonal to ovoid epithelial cells with hepatocyte morphology arranged in small to large sized cohesive clusters. They have variably distinct cellular borders with abundant fine basophilic granular appearing, blue to pink cytoplasm. Low numbers of cells have small amounts of indistinct cytoplasmic vacuolation, and occasional hepatocytes also have a few small, round, clear distinct cytoplasmic vacuoles. Nuclei are round to oval, approximately 1.5x RBC sized, central to eccentrically placed, with fine to coarsely stippled chromatin, and a frequently prominent single nucleolus. Frequent binucleated cells are seen. Anisocytosis and anisokaryosis are mild. Large areas of the slides contain pale basophilic necrotic material and more poorly define hepatocytes, often displaying indistinct cellular detail and poorly preserved nuclei. Frequent dark green granules are found within the hepatocytes, which is likely bile or lipofuscin pigment. Some clusters have dark green to black aggregates of bile suggestive of bile canaliculi casts. Low numbers of neutrophils and small lymphocytes are seen throughout. Rare individual granulated mast cells are seen. No microorganisms are found.

CYTOLOGIC INTERPRETATION:

Mild mixed vacuolar hepatopathy (glycogen and lipid type), with evidence of cholestasis and necrosis.

COMMENTS:

Cytologic findings from these liver aspirates are suggestive of mild mixed vacuolar hepatopathy, with both glycogen and fat vacuoles seen. Mild indistinct vacuolar change/cytoplasmic rarefaction is often a fairly non-specific finding, and is generally associated with glycogen deposition or hydropic degeneration often due to non-specific hepatocellular damage or hypoxia. These changes may be seen with exogenous steroid administration or secondary to increased cortisol, often due to hyperadrenocorticism or stress. It may also be seen as a secondary non-specific change due to nodular hyperplasia, neoplasia, or GI disease. In this case the presence of small numbers of lipid vacuoles raises suspicion for an early lipodosis. The large amount of green lipofuscin pigment is a frequent finding in the hepatocytes of middle-aged to older dogs, and is considered a benign product of cell breakdown and ageing. Bile canaliculi casts suggest cholestasis and impaired bile flow. No obvious neoplastic cells are found, however the possibility of a more well-differentiated hepatocellular carcinoma cannot be entirely excluded. A biopsy and submission for histopathology after coagulation assessment, may be considered in this case.

Guy Davies BSc Vet Path (Hons), BVetMed (Hons), MRSB, MRCVS
Clinical Pathologist

END OF REPORT

Example haematology report:

0200819044827

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START OF REPORT

Patient:		
Access Number:		
ID:		
ASG:	15 Years 4 Months Cat (Feline - Domestic) (Domestic Short Hair) MaleNeutered	
Report Date:		
Date Sample Received:		

Test Results

(HAEM) Routine haematology with profile

(VCBC) Full Blood Count

WBC	L	2.0	$\times 10^9/L$	4.0 - 15.0
RBC	L	2.59	$\times 10^{12}/L$	5.50 - 10.00
Haemoglobin	L	4.5	g/dl	8.0 - 15.0
PCV	L	15.9	%	27.0 - 50.0
MCV	H	61.4	fl	37.1 - 54.7
MCH	H	17.4	pg	11.5 - 16.7
MCHC		28.3	g/dl	28.3 - 34.3
Platelets	L	141	$\times 10^9/L$	200 - 600

Differential		%	Abs	Interval
Neutrophils	L	58 %	$1.1 \times 10^9/L$	2.5 - 12.5
Lymphocytes	L	25 %	$0.5 \times 10^9/L$	1.5 - 7.0
Monocytes		15 %	$0.3 \times 10^9/L$	0.0 - 0.8
Eosinophils		2 %	$0.0 \times 10^9/L$	0.0 - 1.5
Basophils		0 %	$0.0 \times 10^9/L$	

Film :

A small EDTA sample - APPROXIMATE RESULTS ONLY.

Absolute retic count = $10.4 \times 10^9/L$.

Non-regenerative anaemia = less than $60 \times 10^9/L$

Mild regeneration = $60-120 \times 10^9/L$

Moderate regeneration = $120-220 \times 10^9/L$

Marked regeneration = greater than $220 \times 10^9/L$

No clot detected.

Manual PCV checked. Not reported.

Nucleated red blood cells seen.

Anisocytosis ++

Rouleaux +

Agglutination: negative (confirmed by saline agglutination test)

Reactive lymphocytes seen.

Platelets in sample are clumped. Platelet count, above, should be considered a minimum value. Smear estimate appears slightly decreased ($100-200 \times 10^9/L$).

about:blank

Haematology Senior Technician

Significant haematology alterations are present. You may consider requesting a board-certified clinical pathologist haematology case review, including a cytologic evaluation of the blood smear (please quote code PHR [REDACTED]). Please note that requests received after midday cannot be guaranteed to be fulfilled on the same day.

(PHR) Pathologists Haematology Review

Pathologists Haematology Review comments

PATHOLOGISTS HAEMATOLOGY REVIEW

HISTORY:

No clinical history provided.

BLOOD SMEAR EVALUATION:

RBC - Erythrocyte density appears moderately decreased with visible rouleaux and mild erythrocyte anisocytosis. Rare metarubricytes are seen.

WBC - Neutrophil numbers appear markedly decreased with a marked decrease in lymphocyte numbers. Neutrophil morphology appears unremarkable. Lymphocytes are predominantly typical small cells with rare intermediate sized reactive lymphocytes found with increased amounts of deeply basophilic cytoplasm.

PLT - Frequent platelet clumps are seen with rare giant platelets.

No microorganisms or overtly neoplastic cells are found.

INTERPRETATION:

Moderate (macrocytic normochromic) anaemia - non-regenerative.

Marked leukopenia - Marked neutropenia and lymphopenia

Platelet clumps present.

COMMENTS:

The moderate anaemia appears non-regenerative. A non-regenerative anaemia may be due to inflammatory disease, chronic systemic diseases, or bone marrow suppression/disease (e.g. due to drugs, toxins, infections, neoplasia, or myelodysplasia). A pre-regenerative anaemia should also be considered if there is any acute haemorrhage/haemolysis (< 5 days duration) - a Coombs test may be considered.

The marked neutropenia may be due to overwhelming inflammatory demand as a result of severe infectious or inflammatory disease. Other causes include immune-mediated neutrophil destruction, toxins, drug-reactions, bone marrow disease, or the presence of viral or atypical infections. Has FIV/FeLV testing been performed? The marked lymphopenia may be a combination of stress and acute infection/inflammation. Reactive lymphocytes are relatively non-specific and are often seen due to systemic antigenic stimulation.

Platelet clumps are seen throughout the smear which can lead to inaccurate platelet results, therefore the platelet count should be considered a minimum value and a reliable estimate of platelet number cannot be provided.

A repeat CBC in 3-5 days to assess for any regenerative response is advised. Further investigation for any underlying causes is recommended, particularly for any sources of infection/inflammation. A persistent hyperglobulinaemia is noted from the previous sample, which may be due to inflammation (polyclonal) or neoplasia (monoclonal). Serum protein electrophoresis may be considered to help differentiate the underlying process. If no underlying focus of inflammation is found, and the leukopenia/anaemia remains persistent, then a bone marrow aspirate and core biopsy are recommended.

Guy Davies BSc (Vet Path), BVetMed (Hons), MRSB, MRCVS
Clinical Pathology Resident

(INTBLO) Intestinal Blood Test Profile

Sodium

147

mmol/l

140 - 157

about:blank

Potassium	4.3	mmol/l	3.4 - 5.6
Sodium : Potassium	34.19		26.00 - 40.00
Chloride	118	mmol/l	111 - 129
Calcium	2.1	mmol/l	1.6 - 3.0
Phosphate	1.4	mmol/l	0.7 - 2.1
T Protein	H 91	g/l	56 - 81
Albumin	27	g/l	26 - 42
Globulin	H 64	g/l	15 - 57
A:G Ratio	L 0.4		0.6 - 1.4
Urea	10.5	mmol/l	6.1 - 12.5
Creatinine	77	umol/l	45 - 170
CK	139	U/l@37C	0 - 360
Bilirubin	4.4	umol/l	0.0 - 10.0
Cholesterol	2.1	mmol/l	0.9 - 6.5
Bile Acids	5	umol/l	0 - 15
ALT	41	U/l@37C	18 - 77
ALP	11	U/l@37C	11 - 67
Gamma GT	1.0	U/l@37C	0.1 - 9.0
GLDH	0.0	U/l@37C	0.0 - 10.0
Amylase	1226	U/l@37C	200 - 1250
Lipase	17	U/l@37	0 - 25
Triglycerides	0.26	mmol/l	0.20 - 1.30
Glucose	5.3	mmol/l	3.8 - 7.6

Please note: If a serum rather than fluoride oxalate sample has been submitted for glucose measurement, occasionally, serum left in contact with red cells may artefactually lower the glucose value.

Follow on testing canine/feline B12 & Folate or canine TLI, B12 & Folate can be added onto this profile at the top-up price if requested on day of receipt at the laboratory. Please call the lab for further information.

END OF REPORT

about:blank

Example bone marrow cytology and histology report:

20200819055207

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START OF REPORT

Patient:		
Access Number:		
ID:		
ASG:	3 Years 2 Months Dog (Canine - Domestic) (Mountain Dog - Bernese) Male	
Report Date:		KOBENHAVN K
Date Sample Received:		DENMARK
		2450

Test Results

(HISCY) Histology and cytology

Cytology

SAMPLE RECEIVED:

4x slides of bone marrow aspirate. History of anorexia, fever and weight loss over the last 14 days. Bleeding from nose. Coagulation profile normal, no sign of Angiostrongylus. Haematology shows poorly-regenerative anaemia, thrombocytopenia ($34 \times 10^9/L$) and pronounced leukocytosis with either lymphocytosis or monocytosis. Positive Borrelia serology.

MICROSCOPIC DESCRIPTION:

Four bone marrow aspirate smears are of poor diagnostic quality with marked haemodilution and overall adequate cellular preservation. Moderate numbers of haematopoietic cells are seen against a basophilic proteinaceous background with rare clear distinct lipid vacuoles suggesting a hypercellular marrow. No stainable iron is seen throughout the smears. Rare megakaryocytes are seen (one per slide), composed of typical mature forms. A differential count is performed which yields on average 83% myeloid cells (74% early myeloid and 9% late myeloid), and 12% erythroid cells (4% early erythroid, 8% late erythroid), with 5% lymphoid cells. The myeloid series is markedly left shifted with predominantly promyelocytes with fewer myeloblasts found. Blast cells account for approximately 9% of the total nucleated cell count. Very rare myelocytes, metamyelocytes and band neutrophils are seen. Moderate numbers of segmented neutrophils are seen, many of which may be peripheral blood-derived. Maturation of the erythroid series appears orderly and complete. The M:E ratio is markedly increased at 6.9 [RI 0.8 - 2.5]. Rare vacuolated macrophages are seen, occasionally with phagocytosed cellular debris. No microorganisms are seen.

CYTOLOGIC INTERPRETATION:

Poor diagnostic quality - marked haemodilution.

Hypercellular marrow - marked myeloid hyperplasia with left shift and maturation arrest, suggested megakaryocyte hypoplasia.

COMMENTS:

This bone marrow aspirate is unfortunately of poor diagnostic quality due to the marked haemodilution and lack of defined marrow particles, therefore the results should be interpreted with caution. Overall the marrow appears hypercellular with little adipose tissue visible. The cellular findings are consistent with a marked myeloid hyperplasia, which appears significantly left shifted with a maturation arrest at the promyelocyte stage. Low numbers of mature segmented neutrophils are seen which may be blood-derived. The predominance of immature myeloid cells with a depletion of more mature cells may suggest peripheral consumption, seen with acute inflammation; or may suggest a possible developing acute myeloid leukaemia (AML). Only rare megakaryocytes are seen suggesting a megakaryocyte hypoplasia, however this may reflect the poor sample quality and marked haemodilution. The erythroid lineage appears to have complete and orderly maturation and so the poorly regenerative anaemia may be secondary to the myeloid hyperplasia or due to peripheral effects, e.g. anaemia of inflammatory disease.

Further monitoring of peripheral cell counts and examination for any atypical cells in peripheral blood is recommended with submission of EDTA blood for a CBC and haematology review. Investigation for any other sources of infection/inflammation are advised as the positive *Borrelia* serology result may indicate exposure or genuine infection. If no focus of infection or inflammation is found and the leukocytosis/anaemia/thrombocytopenia remain persistent, then a repeat bone marrow aspirate may be considered.

Guy Davies BSc (Vet Path), BVetMed (Hons), MRSB, MRCVS
Clinical Pathology Resident

Histology

SAMPLE RECEIVED:

Bone marrow core biopsy. 1x section of H&E stained bone marrow is submitted.

MICROSCOPIC DESCRIPTION:

The bone marrow section is of good quality and comprises numerous well preserved marrow spaces surrounded by trabecular bone of normal morphology with smooth margins. The overall cellularity appears increased at approximately 90% compared with adipose tissue. Small amounts of stainable iron is seen. Megakaryocytes appear decreased in number overall and appear focally distributed, although they are composed of predominantly mature cells. Myeloid precursors predominate with a marked increase in early precursors which appear relatively monomorphic and evenly distributed throughout the marrow. These cells have round to ovoid shape with round nuclei containing vesicular chromatin and occasional prominent one or two nucleoli. Low numbers of more mature myeloid precursors are seen with a few segmented neutrophils found. Low numbers of erythroid precursors are found, which display an orderly and complete maturation. M:E ratio is markedly increased at approximately 8:1. Rare eosinophils, small lymphocytes and plasma cells are seen. Occasional mitotic figures are found. No microorganisms are seen.

INTERPRETATION:

Hypercellular marrow

Marked myeloid hyperplasia - left shifted - suspicious for acute myeloid leukaemia (AML)

Megakaryocytic hypoplasia

Erythroid hypoplasia.

COMMENTS:

Findings from this bone marrow core biopsy show a hypercellular marrow with a predominance of early myeloid precursors. These cells frequently have a blast cell morphology (which appear increased in number compared with the aspirates) and are most likely myeloblasts or promyelocytes. The prevalence of these cells throughout the marrow raises suspicion for an acute myeloid leukaemia (AML). Low numbers of late stage precursors are seen in this biopsy section. The megakaryocyte and erythroid lines both appear to be reduced in number, likely secondary to the increased myeloid component, however maturation of these lines appears orderly and complete. These findings would account for the peripheral thrombocytopenia and anaemia. Continued monitoring of these lines is advised.

Submission of blood for a CBC and smear review is recommended to assess for the presence of a leukaemic population. Exclusion of any infectious or inflammatory causes of myeloid hyperplasia is also advised. Immunohistochemistry is recommended to rule out possible lymphoid origin of the blast cells (CD3, CD79a, PAX-5).

Please contact the laboratory if you wish to proceed with the immunohistochemistry.

Guy Davies BSc (Vet Path), BVetMed (Hons), MRSB, MRCVS
Clinical Pathology Resident

(AAA) Preliminary Report

Preliminary Report

Sample needs further fixation/longer processing, results to follow ASAP.

END OF REPORT

about:blank

1.4. Personal Caseload Summary

The number of cytology cases and haematology case reviews detailed in Tables 1 and 2 have been completed by me at either Finn Pathologists or Pride Veterinary Hospital (a clinical partner of the University of Nottingham) since November 2017 until July 2020. These represent cases for which cytology or haematology reports have been written by me for clinical diagnostic reporting and/or training purposes. Table 2 shows a more detailed breakdown of cytology cases by both species and sample type.

Table 1. Caseload summary from November 2017 to July 2020, detailed by species. Cases were completed at Finn Pathologists and Pride Veterinary Hospital.

	Canine	Feline	Exotic	Equine/Farm	Total
Haematology	719	360	29	18	1126
Cytology	2492	717	27	7	3243
Histology (bone marrow)	11	2	0	0	13
Urine	237	122	1	0	360
Total	3459	1201	57	25	4742

Table 2. Cytology case load completed from November 2017 to July 2020 detailed by species and sample type. Cases were completed at either Finn Pathologists or Pride Veterinary Hospital.

Sample Type	Canine	Equine/Farm	Feline	Exotic/Small mammal	Total
Abdominal mass	19		39	4	62
Anal/Perianal mass	61		1		62
BAL/Tracheal wash	83	1	61		145
Bile	8		8		16
Bladder	10		1		11
Body cavity effusion	113		141		254
Bone	7		1		8
Bone marrow aspirate	12		3		15
CSF	122		6		128
Cyst fluid	85		20	1	105
Digit mass	12				12
Ear swab	11		2		13
Eye	3		3		6
Intestine	3		22		25
Kidney	4		8		12
Lip mass	14		3		17
Liver	71		29		100
Lung	8		3		11
Lymph Node	334	1	120	1	456
Mammary	64		18		82
Mediastinal mass	2		3		5
Muscle	10		1		11
Nasal flush/smear	21		10	1	31
Nasal mass	2		4		6
Oral mass	28		13		41
Pancreas	4		1		5
Penis mass	1				1
Prostate	18				18
Prostatic wash	48				48
Rectal mass	1				1
Retrobulbar mass	2				2
Salivary gland	8		1		9
Skin masses	1045	5	155	20	1225
Skin scrape/Impression	18		8		26
Spleen	46		10		56
Stomach	8		7		15
Synovial fluid	238		9		247
Testicle	8				8
Thoracic mass	7		3		10
Thyroid	7		3		10
Tongue	3		6		9
Tonsil	3		1		4
Vaginal cytology	11				11
Vaginal mass	5				5
Total	2492	7	717	27	3243

1.5. Regular Seminars

- Journal club – every 2 weeks.
 - Regular discussion between the clinical pathology residents about relevant papers in the core journals in the reading list. The past 5 years of the journal 'Veterinary Clinical Pathology', 'Journal of Veterinary Internal Medicine' and relevant issues of 'Veterinary Clinics of North America' were covered.
- General pathology rounds – monthly.
 - Discussion of a chapter of the core general pathology texts (Robbins and Zachary/McGavin) along with a short quiz, usually with the anatomic pathology residents and one of the anatomic pathologists.
- Supervisor meeting – every week.
 - Regular weekly meeting with Peter Graham to discuss progress with the Masters project, clinical pathology textbook chapter reviews, or interesting/useful cases.
- Cytology/Haematology rounds – weekly.
 - Meeting of the Finn clinical pathology team to discuss interesting cytology/haematology cases. Cases also may have been sourced from the lab archives or personal collections. The meeting was generally led by one of the team, including myself, on a rotating basis.
- ASVCP Online Rounds – monthly.
 - Each month there are cytology and haematology rounds provided usually by ACVP/ECVCP diplomates or senior residents in the form of an online webinar. Usually challenging or unusual cases would be discussed, which often include exotic species or rarer diseases. More common conditions may be discussed

but often with a new presentation or a more advanced diagnostic technique.

The full clinical information around the case, along with accompanying cytology/haematology smears, laboratory data, imaging and clinical findings are discussed with each case.

1.6. Teaching and Outreach Contributions

I contributed to the teaching of final year vet students during my time at Nottingham in the second year. As part of the undergraduate teaching on the Veterinary Medicine course, year-5 students currently undertake a 2-week pathology rotation. This rotation includes training in both anatomic and clinical pathology. The clinical pathology teaching involves discussion of an assigned set of cytology cases, along with cases designed to develop interpretation of haematology and biochemistry lab result data. During the second year I regularly held these sessions myself in the absence of Peter Graham, whilst also attending the feedback session and student rounds on the last Friday of the rotation. At this meeting the students present an interesting case they have seen and give details on post-mortem gross findings, diagnosis, pathogenesis, and any relevant clinical pathology data.

I have made also teaching contributions on the veterinary undergraduate course, assisting with practicals and group seminars to years 1-4 on relevant clinical pathology-related topics, including blood smear and haematology result interpretation, endocrinology result interpretation, renal and urinary pathophysiology, skin cytology, and general cytology. I

taught many of these sessions with fellow residents and in some I assisted various academics in the vet school.

Since being based at Finn Pathologists, I was involved in supervising veterinary students and early career veterinary surgeons who visit the lab as part of undergraduate research projects or as visiting clinicians wishing to gain experience of a commercial laboratory. I also contributed to the company's new graduate programme providing lectures on obtaining appropriate samples for cytology, and lectures on basic blood smear and cytology interpretation in a practice setting.

Since October 2018 I have been the Veterinary Clinical Pathology Trainee Representative for the Royal College of Pathologists. This role involves representing the RCPATH trainees in my field at the Veterinary Pathology Specialty Advisory Committee and the college's trainee committee.

1.7. Conferences, CPD, Placements and Industry events

Date	Description
December 2017	Statistics for Bioscientists , Postgraduate course - University of Nottingham. Tutor – Professor Ian Hardy, AES Dept. [10 training credits]
January 2018	Royal Veterinary College , London - Two-week externship placement to the clinical pathology department.
April 2018	BSAVA Congress - Birmingham. [6 training credits]
19 th & 20 th April 2018	SB Campus PGR Symposium – Presentation of year 1 poster. [7 training credits]
June 2018	Practice exam - IDEXX, Wetherby . Half size mock exam based on combined features of each of the three available clinical pathology board examinations (ACVP/ECVCP/RCPATH). The examination contained sections on each of the four core areas of veterinary clinical pathology – general pathology (including lab management), haematology, biochemistry, and cytology

October 2018	ESVCP Annual Congress – Athens. [6 training credits]
November 2018	Flow cytometry short course - University of Nottingham Medical School. One day course on the basis and clinical application of flow cytometry. Undertaken at the Queen's Medical Centre. [1 training credit]
November 2018 - March 2019	VIN online course - Quality Management in the Veterinary Clinical Pathology Laboratory - Part 1. Organised by members of the ECVCP Lab standards committee. 14-week course covering various aspects of laboratory management including: ISO certification, Six sigma, LEAN production, external quality control and quality assurance, internal quality control and control rules, biologic variation, and laboratory design. Weekly essays and reports were submitted to be graded by an assigned mentor, usually an expert in the field. Assessed by a final written exam at the end of the course.
March 2019	Nottingham Pathology Festival (co-organiser) - Careers day for undergraduate veterinary students highlighting potential career pathways in veterinary pathology. Approximately 90 students attended from most UK vet schools and Dublin vet school. Various speakers attended from pharmaceutical companies, universities and diagnostic laboratories. I gave a short lecture on residency programmes and an overview of specialist training in pathology. I also attended a Q&A panel answering questions regarding pathology training.
April 2019	BSAVA Congress - Birmingham. Oral presentation of clinical abstract in the Canine General Practice stream. Presentation title - <i>Retrospective analysis of Canine Bronchoalveolar Lavage submissions to referral veterinary laboratories in the UK.</i> [5 credits]
2018 - 2019	Nottingham Pathology Society (co-founder) - Society for undergraduate veterinary students interested in veterinary pathology. I contributed to monthly meetings, discussing various cases and clinical pathology topics
June 2019	Finn Pathologists - Histopathology training. One-week placement for training in histopathology with the team at Finn Pathologists. Supervised by Melanie Dobromylskyj (FRCPath). Training focused on the most relevant tissues to clinical pathology correlation such as skin, liver, and haematopoietic/lymphoid tissue; with emphasis on common tumours, patterns of inflammation and toxicity, and types of lymphoma. Some aspects of special stains and immunohistochemistry were also covered.
July and September 2019	Finn Pathologists CPD programme - Use of Immunohistochemistry in the diagnosis of tumours, parts 1 & 2
October 2019	Finn Pathologists - Histopathology training. One-week placement for training in histopathology with the team at Finn Pathologists. Supervised by Melanie Dobromylskyj (FRCPath), Mark Wessels (FRCPath) and Ann Pocknell (DipACVP).
October 2019	Finn Pathologists - Farm animal pathology. Tutored sessions delivered by Mark Wessels (FRCPath) (assisted with cases from James Barnett (FRCPath) at Axiom laboratories). Topics covered included toxicology, common infectious diseases, hepatic disease and farm animal clinical biochemistry.
December 2019 - March 2020	VIN online course - Quality Management in the Veterinary Clinical Pathology Laboratory - Part 2. Organised by members of the ECVCP Lab standards committee. 13-week course continuing from the material covered in part 1 of the course. Topics included: Biologic variation, method validation and comparison, reference intervals, external quality assurance, SOP forms, ISO certification, audits, risk assessment, and safety regulations.
June 2020	ESVCP-ACCP Congress - Vienna (changed to online format due to the COVID-19 pandemic). The general focus of the congress was toxicologic pathology and equine clinical pathology. [4 training credits]
September 2020	RCPATH Veterinary Pathology Trainee Summer School. Online conference. [2 training credits].
	Total training credits (conferences and internal courses) - 41

Three publications are currently being prepared based on data obtained from the research project. The first is a draft manuscript from the method comparison study titled '*Evaluation of a point-of-care quantitative dry-chemistry assay for measurement of canine c-reactive protein*' - which was written during the final year of the residency programme and is due to be submitted within two months of completing the programme. Two further draft papers from each of the prospective CRP BAL study and retrospective BAL data were written during the final year of the residency.

Chapter 2

Research Project

Feasibility of using point-of-care c-reactive protein assays in the diagnosis of canine lower respiratory tract infections - a basis for targeted antibiotic use?

2. Literature Review

2.1 C-reactive Protein (CRP) and the Acute Phase Response

C-reactive protein (CRP) is one of the acute phase proteins, a group of proteins produced primarily by the liver that form part of the innate immune response. The acute phase response occurs shortly after tissue injury or infection, and can be stimulated by a range of inflammatory conditions, including trauma and neoplasia (Eckersall and Bell, 2010). It is non-specific and forms a rapid reaction, often before the onset of clinical signs, and therefore may be considered as an early marker of any inflammatory pathologic process (Cerón *et al.*, 2005).

Acute phase proteins (APP) are generally grouped into four categories according to the magnitude of their response to stimuli. They are categorised as positive APPs – and then either: major, moderate, minor positive APPs; or they are negative APPs (Cerón *et al.*, 2005; Cray *et al.*, 2009; Eckersall and Bell, 2010). Major positive APPs generally have a low baseline serum concentration but increase 10 to 100-fold. Moderate APPs increase by 2 to 10-fold, and minor APPs increase by a slight amount or 2-fold (Cray *et al.*, 2009). Negative APPs decrease in blood concentration during the acute phase response. The primary negative APP in most species is albumin, thought to be due to reduced synthesis by the liver, allowing for amino acids to then be utilised for synthesis of positive APPs and other inflammatory mediators (Eckersall, 2008; Paltrinieri, 2008). Transferrin has also been shown to be a negative APP (Cerón *et al.*, 2005).

The classification of APPs varies between species [Table 3]. C-reactive protein is constitutively expressed in cats and cattle and therefore has little clinical use (Kajikawa *et al.*, 1999; Petersen *et al.*, 2004; Paltrinieri, 2008). Serum amyloid A (SAA) is unique in being a major acute phase protein in humans and all studied domestic species, except for the rat (Uhlar and Whitehead, 1999).

Table 3. Classification of major and moderate acute phase proteins in various species.

Species	Major APP	Moderate APP	References
Dog	CRP, SAA	Hp, Cp, α 1-AGP, Fb	(Martínez-Subiela <i>et al.</i> , 2003; Murata, <i>et al.</i> , 2004; Cerón <i>et al.</i> , 2005; Eckersall, 2008)
Cat	SAA, α 1-AGP	Hp, Cp	(Kajikawa <i>et al.</i> , 1999; Cerón <i>et al.</i> , 2005; Paltrinieri, 2008)
Horse	SAA	Hp, Fb, CRP, D-dimer	(Jacobsen <i>et al.</i> , 2006; Jacobsen, Thomsen and Nanni, 2006; Jacobsen and Andersen, 2007; Armengou <i>et al.</i> , 2008; Zabrecky <i>et al.</i> , 2015; Nikvand <i>et al.</i> , 2019)
Cow	SAA, Hp	Cp, α 1-AGP, Fb	(Jacobsen <i>et al.</i> , 2004; Cray <i>et al.</i> , 2009)
Pig	SAA, MAP, Hp	CRP, Fb	(Lampreave <i>et al.</i> , 1994; Heegaard <i>et al.</i> , 1998; Murata <i>et al.</i> , 2004)
Human	CRP, SAA	Hp, α 1-AGP, Fb	(Volanakis, 2001; Cray <i>et al.</i> , 2009)

CRP – C-reactive protein, SAA – Serum Amyloid A, Hp – Haptoglobin, Cp – Ceruloplasmin, α 1-AGP – α 1-acid glycoprotein, Fb – Fibrinogen, MAP – Major acute-phase protein.

C-reactive protein was first isolated from patients with *Pneumococcus* infections, and thus named for its ability to bind to the C-polysaccharide component of the bacteria (Tillett and Francis, 1930). Canine CRP was first isolated and characterised by (Riley and Coleman, 1970), and subsequently further characterised by various groups ((Caspi *et al.*, 1984); (Yamamoto *et al.*, 1992)(Yamamoto, Miyaji, *et al.*, 1993); (Eckersall and Conner, 1988)).

2.2 Biochemistry and structure of CRP

C-reactive protein in all species is a pentraxin composed of five subunits, each around 20 kDa in size, with a ligand binding site on each subunit [Figure 1] (Caspi *et al.*, 1984; Thompson *et al.*, 1999). Canine CRP has a similar structure to human CRP but does not share common antigenicity, likely due to the differing glycosylation of the CRP subunit proteins. Two of the five subunits are glycosylated in canine CRP, whereas none are glycosylated in human CRP (Caspi *et al.*, 1984). This is likely to be a factor in the variable cross-reactivity between human CRP immunoassays for detection of canine CRP in serum (Yamamoto *et al.*, 1992, 1993; Kjelgaard-Hansen *et al.*, 2008).

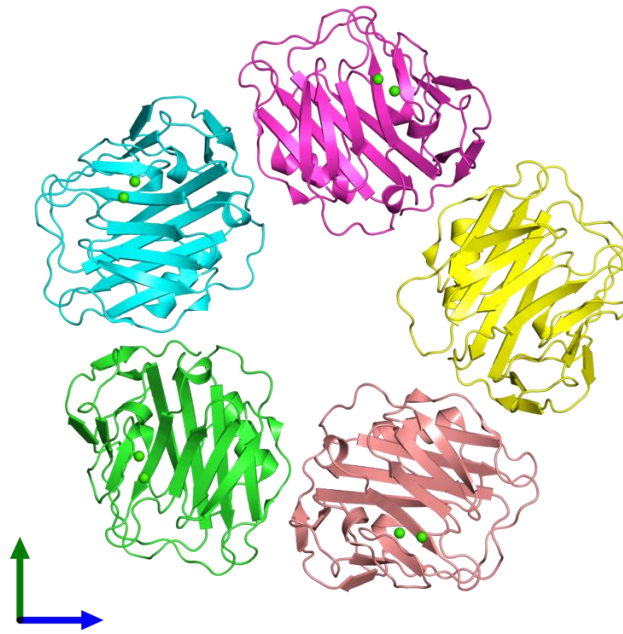


Figure 1 – The molecular structure of human C-reactive protein represented as a ribbon diagram. There are five subunits, each with binding sites for ligands. Here the ligand-binding sites highlighted with calcium ions, represented as green balls. Sourced from the Protein Data Bank in Europe – code 1gnh [<https://www.ebi.ac.uk/pdbe/entry/pdb/1gnh>]. Structure diagram adapted from original source publication - (Shrive *et al.*, 1996).

2.3 Physiology of CRP

In many species, CRP has a wide-ranging role in various physiological processes, centred around providing an innate immune response to pathogens and foreign molecules (Cerón *et al.*, 2005). These processes are summarised in Figure 2. Some functions however, are still poorly understood. The main outcomes and functions of CRP is to induce downstream cytokines, inhibit chemotaxis of neutrophils, and promote the binding of complement, when bound to bacteria, thereby facilitating phagocytosis of bacteria (Mold *et al.*, 2001; Murata *et al.*, 2004b). In humans CRP has been shown to both bind-to and modulate neutrophils (Buchta *et al.*, 1987a; Buchta *et al.*, 1987b). It is thought to bind through Fc receptors and

promote neutrophil phagocytosis. However, at a high concentration it is thought to have more of a negative feedback role, regulating leukocyte infiltration at inflammatory sites and inhibiting the respiratory burst of neutrophils (Buchta *et al.*, 1987a; Zhong *et al.*, 1998). It binds to its various ligands in a largely calcium-dependent manner (Caspi *et al.*, 1984; Fujise *et al.*, 1992). CRP is predominantly produced in the liver, although there is also some evidence of CRP production in the human and canine kidney (Jabs *et al.*, 2003; Raila *et al.*, 2011). The secretion of CRP has been shown to be based on *de-novo* synthesis with no presence of CRP within hepatocytes in normal homeostasis (Kushner and Feldmann, 1978). Production of CRP is induced by various pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, and TNF α , which are produced by a range of cells including endothelial cells, fibroblasts, and monocyte/macrophage-derived cells (Yamashita *et al.*, 1994; Zhong *et al.*, 1998; Petersen *et al.*, 2004).

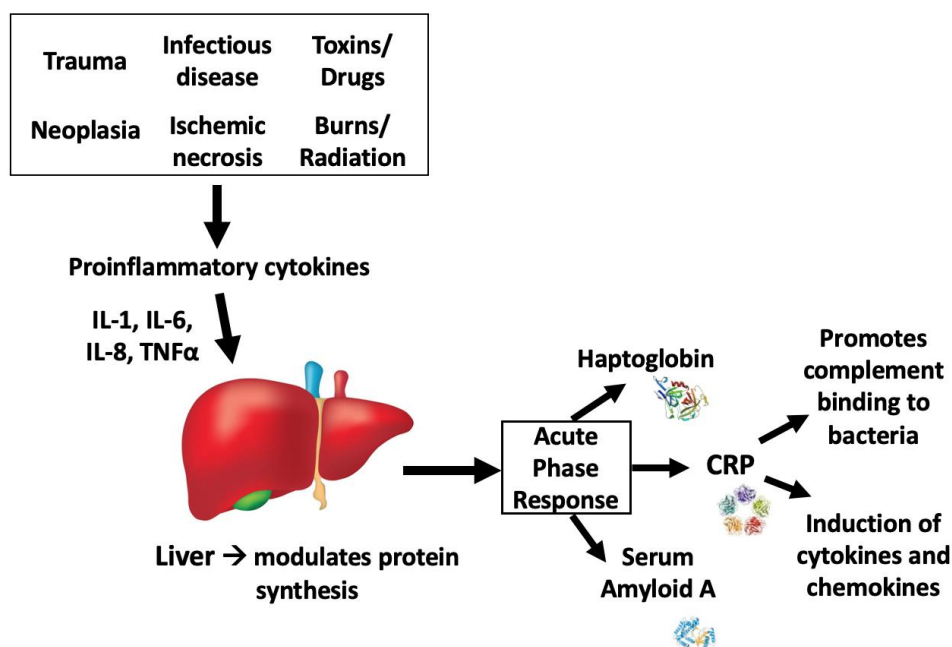


Figure 2 – The acute phase response in dogs with effects of CRP production. Adapted from Cray *et al.*, 2009.

2.4 Clinical use of CRP in dogs

C-reactive protein is characterised by low concentrations in a healthy dog, usually 0 - 20 mg/L (mean 3.65 ± 1.4) (Otabe *et al.*, 1998; Gabay and Kushner, 1999). The normal CRP concentration varies between individuals, but has generally been found to be constant within healthy individuals, without any circadian influence (Yamamoto *et al.*, 1994a; Otabe *et al.*, 1998). One study has reported a higher normal CRP concentration in Miniature Schnauzers (0 - 18.2 mg/L) compared with the normal range of dogs of other breeds (0 – 10.7 mg/L, median 0.1 mg/L) (Wong *et al.*, 2011). Slightly higher levels have also been reported in female dogs, and CRP has been shown to increase during pregnancy, peaking at 30-45 days after ovulation (Kuribayashi *et al.*, 2003). The concentration of CRP generally reaches its peak within 24 hours, following a 4-hour lag phase, after which they normalise quickly after the stimulus has resolved, with canine CRP also shown to have a short half-life (Conner *et al.*, 1988; Yamashita *et al.*, 1994; Eckersall, 2008).

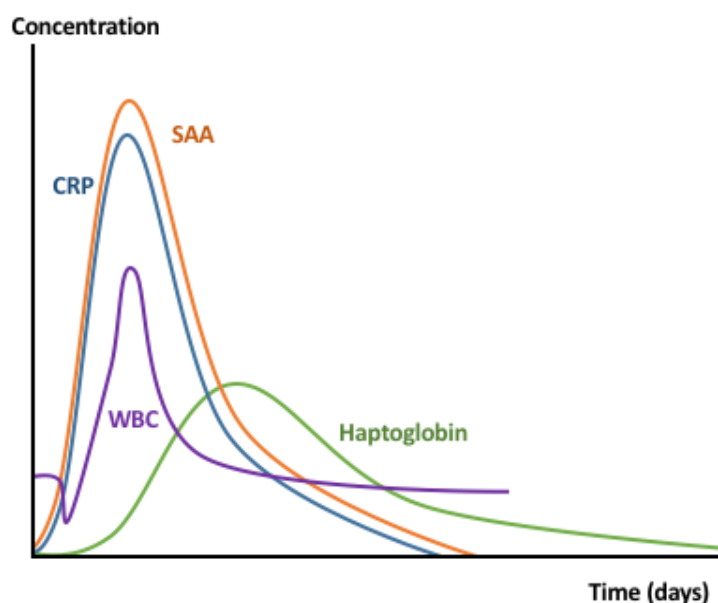


Figure 3 – Diagram of kinetics of different acute phase proteins in dogs, compared with leukocytes. Adapted from Kjelgaard-Hansen and Jacobsen, 2011.

Studies have shown that CRP is significantly increased, and therefore useful in detecting a range of inflammatory conditions, with some evidence that it is a more sensitive predictor of inflammation than white blood cell (WBC) count (Eckersall and Conner, 1988; Nakamura *et al.*, 2008). CRP also has the advantage of being stable in plasma or serum for longer periods compared with WBC (Kjelgaard-Hansen *et al.*, 2003; Hartweg *et al.*, 2007). Whereas WBC are involved in extravasation and localization to the site of inflammation or infection, the acute phase proteins, including CRP, are consistently high throughout any inflammatory stimulus. The properties and kinetics of CRP therefore lend it for use as a rapid marker of inflammatory disease (Cerón *et al.*, 2008). Moderate APPs such as haptoglobin increase slowly and decrease more slowly, therefore making them potentially less suitable for detecting and monitoring acute disease (Eckersall and Bell, 2010; Viitanen *et al.*, 2017). These differences are shown in Figure 3. Some evidence is available to show that Serum Amyloid A (SAA) may be a more rapid and reliable marker for inflammation compared with CRP, however few assays are available for canine SAA, and previous studies in the field of respiratory conditions in humans have predominantly concentrated on CRP as a primary marker (Christensen *et al.*, 2014).

Multiple studies have been published evaluating the use of CRP in canine patients and demonstrating an elevated CRP concentration in conditions of various types, including infectious, neoplastic, inflammatory/immune-mediated, and traumatic causes. Examples of these are listed in Table 4.

Table 4. List of example conditions with increased CRP concentration in dogs.

Cause/Stimulus	References
Infections/Sepsis	
Bordetella bronchiseptica	(Yamamoto <i>et al.</i> , 1994b)
Pyometra	(Dabrowski <i>et al.</i> , 2007; Fransson <i>et al.</i> , 2007)
SIRS	(Fransson <i>et al.</i> , 2007; Torrente <i>et al.</i> , 2015)
Acute pancreatitis	(Mansfield <i>et al.</i> , 2008)
Dirofilaria immitis	(Carretón <i>et al.</i> , 2014; Méndez <i>et al.</i> , 2014)
Babesia spp.	(Milanović <i>et al.</i> , 2017; Ulutas <i>et al.</i> , 2005)
Canine Parvovirus	(Kocaturk <i>et al.</i> , 2010)
Ehrlichiosis	(Mylonakis <i>et al.</i> , 2011)
Leishmania spp.	(Martínez-Subiela <i>et al.</i> , 2002; Cantos-Barreda <i>et al.</i> , 2018; Pardo-Marin <i>et al.</i> , 2020)
Spirocerosis	(Mukorera <i>et al.</i> , 2011; Mylonakis <i>et al.</i> , 2012; Nivy <i>et al.</i> , 2014)
Giant Kidney Worm	(Schmidt <i>et al.</i> , 2016)
Septic arthritis	(Hillström <i>et al.</i> , 2016)
Demodicosis	(Salem <i>et al.</i> , 2020)
Leptospirosis	(Caspi <i>et al.</i> , 1987; Yamamoto <i>et al.</i> , 1993; Buser <i>et al.</i> , 2019)
Neoplasia	
Multicentric lymphoma	(Nielsen <i>et al.</i> , 2007; Mischke <i>et al.</i> , 2007)
Malignant cancer (various)	(Selting <i>et al.</i> , 2015, 2016)
Surgery/Trauma	
Brachycephalic obstructive airway syndrome (BOAS)	(Planellas <i>et al.</i> , 2012)
Gastric mucosal injury	(Otabe <i>et al.</i> , 2000; Bayramli and Ulutas, 2008)
Acute abdomen	(Galezowski <i>et al.</i> , 2010)
Gastric dilatation-volvulus (GDV)	(Israeli <i>et al.</i> , 2012)
Abdominal soft tissue surgery	(Kjelgaard-Hansen <i>et al.</i> , 2013)
Ovariohysterectomy	(Dabrowski <i>et al.</i> , 2007; Kum <i>et al.</i> , 2013)
Degenerative/Inflammatory disease	
Inflammatory bowel disease	(Jergens <i>et al.</i> , 2003)
Steroid-responsive meningitis arteritis (SRMA)	(Bathen-Noethen <i>et al.</i> , 2008; Lowrie <i>et al.</i> , 2009; Biedermann <i>et al.</i> , 2016)

Immune-mediated haemolytic anaemia (IMHA)	(Griebsch <i>et al.</i> , 2009; Mitchell <i>et al.</i> , 2009)
Periodontal disease	(Rawlinson <i>et al.</i> , 2011)
Cystitis	(Seo <i>et al.</i> , 2012)
Congestive heart failure	(Reimann <i>et al.</i> , 2016)
Immune-mediated polyarthritis	(Kjelgaard-Hansen <i>et al.</i> , 2006; Foster <i>et al.</i> , 2014)
Chronic kidney disease	(Raila <i>et al.</i> , 2011)
Pemphigus foliaceus	(Severo <i>et al.</i> , 2018)

2.5 Infectious respiratory diseases in dogs

Respiratory diseases are often grouped by anatomical origin as either upper or lower respiratory tract diseases. Upper respiratory tract infections in dogs are often considered of multifactorial aetiology, often with a viral trigger and occasionally secondary bacterial colonisation, they are commonly grouped as Canine infectious respiratory disease complex (CIRD) (Hillström *et al.*, 2016). Lower respiratory tract infections may be classified as pneumonia, bronchopneumonia, or infectious bronchitis, depending on the tissues affected. Bacterial pneumonia is defined as an inflammation of the lower airways and lung parenchyma as a result of bacterial infection, either due to initial viral infection followed by bacterial colonisation, aspiration or foreign body contamination (Dear, 2014). Some evidence suggests that infectious community-acquired pneumonia is more common in younger dogs, compared with aspiration or opportunistic infections secondary to underlying disease in older animals (Radhakrishnan *et al.*, 2007).

2.6 Respiratory disease cytology and airway sampling in dogs

Cytologic and microbiological examination of airway fluids have been routinely used as a means of diagnosing lower respiratory tract infections in veterinary and human medicine. In

canine patients, available methods include tracheal aspiration (TA), transtracheal wash (TTW) or bronchoalveolar lavage (BAL), however TA and TTW are less frequently used, likely because the trachea and carina are not sterile even in healthy dogs (Dear, 2014). BAL has been shown as the preferred method for diagnosing lower respiratory tract disease due to the sampling of smaller airways, alveoli and interstitial tissues, whereas the other methods are purely upper airway samples (Hawkins *et al.*, 1995). BAL has also been shown as the best method for diagnosing bacterial and fungal airways diseases in particular (Hawkins and DeNicola, 1990). One limitation of all airway washes, and in particular BAL samples due to the sampling technique, is the risk of microbial contamination as the upper respiratory tract and oropharynx are not considered sterile sites. This may lead to false-positive culture results and resulting in an inappropriate use of antimicrobial drugs (Johnson *et al.*, 2013). Cytologic criteria have been used to identify potential contaminated samples, such as an increased percentage of squamous epithelial cells present, or the presence of *Simonsiella* oropharyngeal commensal bacteria (Peeters *et al.*, 2000). A previous study of BAL samples in dogs with respiratory disease showed that the presence of intracellular bacteria on cytology, along with a positive quantitative culture of 1.7×10^3 colony-forming units per millilitre of BAL fluid, indicates a high likelihood of clinically relevant lower respiratory tract infection (Peeters *et al.*, 2000). However, quantitative culture is not always completed and many diagnoses of respiratory infection are often made with any positive culture and supportive clinical signs (Angus *et al.*, 1997; Radhakrishnan *et al.*, 2007).

Bacterial respiratory infections are most often diagnosed after finding septic suppurative inflammation on cytology, i.e. degenerate neutrophils with intracellular bacteria and an overall increased cellularity (Johnson *et al.*, 2013). Cytology from multiple lobes can reveal

different findings, and may often lead to an inflammatory population without evidence of sepsis (Epstein, Mellema and Hopper, 2010). This can often be the case with more localized pneumonia or infectious diseases, or with aspiration or foreign body-induced pneumonia (Dear, 2014). Non-infectious diseases may include haemorrhagic or neoplastic diseases, or various inflammatory conditions, which can be characterised by the predominant inflammatory population on cytology. Inflammation in the airways can be classified as acute neutrophilic, chronic, mixed, or eosinophilic, each of which suggest specific disease processes. For example, an increased number of eosinophils may be indicative of allergic disease, or goblet cell hyperplasia and increased mucus with a mixed inflammatory pattern may be seen in chronic bronchitis (Hawkins and DeNicola, 1990; Burkhard and Millward, 2010).

Normal cytological findings in airway washes vary greatly between studies, potentially partly due to the variable methods used, but a summary of a consensus between studies is shown in Table 5. The predominant cell type in normal washes is macrophages, which may or may not be activated, with smaller numbers of respiratory epithelial cells and other leukocytes, as well as variable amounts of mucus (Rebar, DeNicola and Muggenburg, 1980; Hawkins and DeNicola, 1990; Hawkins, DeNicola and Plier, 1995; Vail, Mahler and Soergel, 1995; Johnson *et al.*, 2013).

Table 5. Expected cell count percentage ranges for cells seen in bronchoalveolar lavage samples in healthy dogs. Adapted from (Burkhard and Millward, 2010).

Cell Type	Percentage Range
Total Cell Count [cells/ μ L]	<500
Macrophage	70-80%
Lymphocyte	6-14%
Eosinophil	<5%
Neutrophil	<5%
Mast Cell	1-2%
Mucus	Minimal to moderate amounts
Ciliated columnar epithelial cells	Few
Cuboidal epithelial cells	Few
Goblet cells	Rare

Microbiological culture should be performed on all airway wash cases, and should include aerobic and anaerobic culture, and ideally culture for *Mycoplasma spp.* (Chalker *et al.*, 2004; Jameson *et al.*, 1995; Chalker, 2005; Rycroft, Tsounakou and Chalker, 2007). Mixed infection is common in adult dogs, but there are mixed findings from juveniles (Jameson *et al.*, 1995; Radhakrishnan *et al.*, 2007). Increases in antimicrobial resistance has also been found in hospital and ventilation-acquired pneumonias in dogs (Epstein, Mellema and Hopper, 2010). Diagnosis of clinically significant infectious disease can still be a challenge. Cytologic evidence of intracellular bacteria is not always found even with significant positive microbial culture, and a lack of organisms isolated on culture with concurrent positive cytologic evidence of sepsis is also possible (Angus, Jang and Hirsh, 1997; Johnson *et al.*, 2013). This may be due to poor sampling technique, death of organisms in transport, or poor fluid sample processing.

2.7 Use of CRP in human medicine for community-acquired pneumonia

C-reactive protein is used as a tool to aid in the diagnosis of community-acquired pneumonia in human medicine (Smith and Lipworth, 1995; Smith *et al.*, 1995; Lim, Smith, Wise, Welham, S. V Baudouin, *et al.*, 2015). This has led to some reduction in the prescription of antibiotics in general practices without necessarily increasing adverse patient outcomes (Eccles *et al.*, 2014; Bates *et al.*, 2017). The UK NICE guidelines for community-acquired pneumonia detail the use of CRP in aiding diagnosis of this condition (National Institute for Health and Care Excellence, 2014). The British Thoracic Society guidelines give similar recommendations (Lim *et al.*, 2009). Uptake of CRP as a routine diagnostic test for community-acquired pneumonia across UK general practices has been variable, with various barriers reported, although these barriers are often at a regional or national finance level rather than being unsupported by clinicians or disrupting the workflow of practices (Huddy *et al.*, 2016).

The NICE guidelines for community acquired pneumonia in people detail 3 pathways for action depending on the results of the CRP assay (National Institute for Health and Care Excellence, 2014):

- Do not routinely offer antibiotic therapy if the C-reactive protein concentration is less than 20 mg/litre.
- Consider a delayed antibiotic prescription (a prescription for use at a later date if symptoms worsen) if the C-reactive protein concentration is between 20 mg/litre and 100 mg/litre.

- Offer antibiotic therapy if the C-reactive protein concentration is greater than 100 mg/ litre.

This guideline enables general practice clinicians to determine if antibiotic therapy is genuinely needed in conjunction with other clinical decision frameworks at the primary point of care, based on the patient's acute phase response and clinical signs. The drawback with this method, and also with its translation into veterinary medicine, is that a diagnosis of bacterial pneumonia may be made (and subsequent antimicrobial therapy for that condition prescribed) when other causes of CRP increase may be possible. Conditions such as some neoplastic diseases, immune-mediated haemolytic anaemia, pyometra, renal disease and sepsis, along with many others have been shown to significantly increase serum CRP levels compared with normal reference intervals (Mischke *et al.*, 2007; Nielsen *et al.*, 2007; Griebisch *et al.*, 2009; Mitchell *et al.*, 2009; Raila *et al.*, 2011; Dabrowski, Kostro and Szczubiał, 2013; Torrente *et al.*, 2015; Selting *et al.*, 2016). However, one benefit of using CRP in patients that have high concentrations at diagnosis, is the possibility of using repeat assays as a tool for monitoring therapy and guiding removal of antimicrobial therapy at an appropriate time (Shaddock, 2016).

2.8 Analytical methods used to measure CRP

Methods used to analyse CRP levels in dogs are generally immunological methods. Many are assays adapted from use in human cases, whereas others are developed specifically for canine use. Early methods that were developed, and then trialed in canine patients were generally latex agglutination, Enzyme-linked immunosorbent assays (ELISA), or immunoturbidometric assays; although most of these early tests were assessed in a

research context and not commercially produced (Eckersall, Conner and Parton, 1989; Eckersall, Conner and Harvie, 1991a; Yamamoto *et al.*, 1994a; Tagata *et al.*, 1996). One of the earliest canine specific studies used immunodiffusion to isolate and quantify CRP in canine serum, but this has not been developed into a marketed diagnostic assay (Yamamoto *et al.*, 1992).

Further evaluation of human immunoturbidometric (IT) (*Bayer*) and canine ELISA methods were completed by Kjelgaard-Hansen *et al* in 2003, which were some of the earliest studies of commercial assays in dogs. These studies showed that the two methods could reliably be used in canine patients, although the canine-specific ELISA method showed poor precision, which may have been of minimal consequence given the assay was still able to significantly differentiate dogs with infections from healthy dogs or those suffering from other diseases (Kjelgaard-Hansen, Jensen and Kristensen, 2003; Kjelgaard-Hansen, Kristensen and Jensen, 2003). The IT assay showed adequate precision and reliability in measuring canine CRP for diagnostic purposes (Kjelgaard-Hansen, Jensen and Kristensen, 2003), although one issue with using assays developed for human use in canine patients is the variable cross-reactivity of CRP with anti-CRP antibodies between species (Yamamoto, Miyaji, *et al.*, 1993). In contrast one study showed that two human CRP assays (one a turbidometric latex assay, another a chemiluminescent immunoassay) failed to reliably detect canine CRP, and were of no use in canine patients (Fransson, Bergström, *et al.*, 2007).

The first rapid point-of-care assay developed commercially for canine patients (*CRP Rapid Assay Pack, EVL*) was one based on immunochromatography, where test strips showed a negative, positive or weak-positive result (McGroddy *et al.*, 2004). The test was compared

with a previously developed ELISA (Eckersall et al., 1989) and differentiated 'positive' results as those over 11 mg/L. Weak positive results were considered similar to negative results, which enabled reliable assessment of increased CRP levels. Although this is a rapid patient-side test, the semi-quantitative nature of the results limits its wider use.

A canine-specific time-resolved immunofluorometric assay (TR-IFMA) has been developed and validated for use in both canine serum and canine and porcine whole-blood samples (Parra *et al.*, 2005, 2006; Martinez-Subiela *et al.*, 2007). These tests are highly sensitive and precise, and have the advantage of being used in both serum and whole-blood samples, but are not yet commercially available in the veterinary market.

A human CRP latex agglutination point-of-care test (*Randox*) has been validated for use in canine patients, and has shown reliable results (Kjelgaard-Hansen, Stadler and Jensen, 2008). The test is a qualitative test and gives a positive or negative result on the test strip. Three further human IT assays have also been evaluated for use in detecting CRP in dogs (*Randox*, *Thermo*, and *Wako*). These reliably detected canine CRP, but precision needed improvement before implementing their use in canine patients (Klenner, Bauer and Moritz, 2010).

In recent years there has been an expansion of new canine CRP tests (both automated and point-of-care assays) developed, many of which have been validated and compared in rigorous method comparison studies (Plickert *et al.*, 2011; Ibraimi *et al.*, 2013; Hillström *et al.*, 2015; Jasensky *et al.*, 2015; Muñoz Prieto *et al.*, 2017a; Piñeiro *et al.*, 2018), often following published veterinary method comparison guidelines (Jensen and Kjelgaard-

Hansen, 2006). Most of these tests have shown excellent correlation with standard methods, but concerns regarding interchangeability between point-of-care tests has been raised (Jasensky *et al.*, 2015). None of these studies have assessed the use of assays in a particular disease situation. This is in contrast to numerous human studies which have recently centred on the use and validation of CRP in community-acquired pneumonia and cardiovascular disease (Hohenthal *et al.*, 2009; Van Vugt *et al.*, 2013; Minnaard *et al.*, 2015).

For most assays, haemoglobin, bilirubin, lipid and anticoagulants (heparin, EDTA, and citrate) have all been shown to interfere with CRP results (Martínez-Subiela and Cerón, 2005). However, with the exception of bilirubin, this has been refuted in a further study by (Hillström *et al.*, 2014). Corticosteroids, both parenteral and orally administered, have been shown to have no significant effect on CRP concentrations in dogs (Martínez-Subiela, Ginell and Cerón, 2004) and there is some evidence that prior antimicrobial usage also has no effect on CRP concentration in both dogs and humans (Bafadhel *et al.*, 2011; Viitanen *et al.*, 2014).

2.9 Application of CRP in cases of lower respiratory disease in dogs

In veterinary medicine, CRP has been shown to increase in various canine respiratory diseases, including bacterial tracheobronchitis and bacterial pneumonia. In an observational study (Viitanen *et al.*, 2014), dogs with bacterial pneumonia had a significantly higher level of CRP compared with other cardiorespiratory conditions (such as chronic bronchitis, canine idiopathic pulmonary fibrosis, and cardiogenic pulmonary oedema), and in turn compared with healthy dogs. The study was performed on a relatively small number of patients (22 dogs with pneumonia), but did benefit from the comparison with healthy control dogs

(n=72), selected from a population of blood donor dogs. The study concluded that CRP could be used as a biomarker for bacterial pneumonia, given the significant increase in these patients (median of 121 mg/l) compared with cases of bacterial tracheobronchitis (median 23 mg/l), or healthy dogs (median 14 mg/l). Other conditions had median CRP values ranging from 5 to 19 mg/l. Given the findings of this study by Viitanen and others, CRP may be used to differentiate cases of bacterial pneumonia from other respiratory cases, but it is unable to distinguish between other cardiorespiratory diseases.

An earlier study has also shown an increase in CRP in dogs with bacterial pneumonia (Nakamura *et al.*, 2008), although this study only reported a modest increase in these cases with a median value of 33 mg/l (ranging from 2-110 mg/l). Some other infectious or inflammatory conditions that were investigated yielded greater increases in CRP compared with normal healthy dogs, these included pyometra, acute pancreatitis, and immune-mediated haemolytic anaemia; Along with various neoplastic conditions such as haemangiosarcoma and nasal adenocarcinoma. This study by Nakamura and others (2008) was conducted as a prospective study on patients at a veterinary referral hospital, and although the total number of patients in the study was high – 928 cases, the number of patients with each individual diagnosis in the study was relatively small. Sixteen cases diagnosed with bacterial pneumonia were assessed. This study did not elaborate further on the potential clinical use of CRP, except to infer that it could be used to differentiate some inflammatory and neoplastic diseases not detected in the complete blood count, and that further work was needed to enable its use as a prognostic factor and variation with clinical severity.

One study has assessed the use of CRP in monitoring response to treatment in cases of bacterial pneumonia in dogs. The study by Viitanen *et al.*, 2017 showed that CRP (along with another acute phase protein – Serum Amyloid A [SAA]) was significantly increased in cases of bacterial pneumonia compared with healthy control dogs. Cases were admitted to a veterinary referral hospital and diagnosed with bacterial pneumonia based on a combination of clinical signs and radiographic findings. CRP and SAA decreased rapidly after initiation of successful treatment, and the reduction and normalisation of CRP levels was used to guide cessation of antimicrobial therapy. The study yielded no adverse effects using this method. This study did draw conclusions as to the possible utility of CRP as a marker for severity of disease (and prognosis in human medicine), but no significant differences were seen in CRP values between short and long-term hospitalised patients (<2 days or >2 days), i.e. patients with differing degrees of severity and treatment response.

C-reactive protein could be used in veterinary medicine in a similar way to that in human medical practice - to aid in the reduction of unnecessary antibiotic prescriptions in a variety of conditions (Lim, Smith, Wise, Welham, S. V Baudouin, *et al.*, 2015). This could potentially promote a decrease in the risk of antibiotic resistance development, as well as avoid potential adverse drug reactions.

This Masters project focuses on lower respiratory tract infections as a model of disease, but the future implications for using CRP as a marker for infectious disease and therefore appropriate prescription of antibiotics is potentially far reaching, and may include cases of cystitis and urinary tract infections, meningitis, sepsis and SIRS, as well as a myriad of other inflammatory conditions.

Given the range of conditions that have been shown to increase CRP levels, and the relatively rapid nature of change in CRP levels in disease, its measurement would be most appropriately implemented at a point-of-care general practice level for differentiating cases where antibiotic therapy is truly needed in patients with suspected conditions such as bacterial pneumonia from those that have a mild self-limiting infection, or those that may resolve with symptomatic treatment alone. This could then enable a reduction in unnecessary antibiotic prescriptions. For example, cases with a suspected lower respiratory tract infection could have a point of care CRP assay performed and if this result showed a concentration within a reference interval, or only a modest increase, then a mild self-limiting infection, or purely inflammatory/non-infectious process may be suspected. This patient would then be recommended symptomatic treatment and be reassessed at an appropriate interval. However, if the CRP level was significantly increased then antimicrobial therapy could be recommended.

2.10 Project Overview

This project was part of a collaboration between CVS Laboratories and CVS Small Animal Clinical Practices with an aim to integrate the use of CRP and other acute phase proteins in current clinical practice, and further investigate their potential use. The initial model used for this purpose was cases of lower respiratory disease infection, and specifically, it was investigated as to whether CRP could be used as a clinical decision tool for guiding antibiotic prescription in cases of lower respiratory tract infection.

To investigate this, serum CRP samples were compared against respiratory microbiological culture and cytology in cases of lower respiratory tract disease. It was hypothesised that the correlation of CRP levels with cytologic and microbiological diagnosis would lead to the formulation of a reference interval and possible decision limit for ruling out infectious disease in lower respiratory cases. It would therefore hopefully be possible to formulate a guideline to use for decision making in these cases, similar to the guidelines used for community-acquired pneumonia in human patients.

In addition, canine CRP is available as an in-clinic test as well as a referral lab one, giving the advantage of a short (often less than 10 minutes) time to result, making it highly suitable for patient-side decision making, including decisions on antibiotic prescription. As a result, an additional aim was to perform a method comparison study to compare the results and possible application of a point-of-care test with an automated referral one. The Gentian cCRP assay on the Beckman Coulter AU680 was used as a reference 'referral' test compared

with the Fujifilm CRP dry-chem slide (vc-CRP-P) assay on the Fujifilm DRI-CHEM NX500 as the point-of-care test.

Prior to commencing the prospective study, some retrospective epidemiological data from all of the CVS Laboratories was collected to investigate the current frequency and distribution of BAL submissions and related microbiological culture submissions; and also the types of diagnosis currently being made from these samples. It was hoped that this would enable the targeting of our prospective data collection and also gain a further understanding as to what current clinical practice for these cases is. This would therefore hopefully give a better framework for creating subsequent guidelines and recommendations for using CRP in these patients.

The study is therefore split into three parts:

1. Analysis of epidemiological data from retrospective BAL cases submitted to the diagnostic laboratories.
2. A prospective CRP study to compare serum CRP concentrations with cytological and microbiological findings in airway washes.
3. Comparison of automated referral and point-of-care assays for analysis of canine CRP.

2.11 Aims of the study

The aims of the project were:

- To assess if CRP can be used to differentiate cases of lower respiratory tract infections in dogs, and to determine appropriate prescription of antimicrobials in these cases.
- To ascertain whether a quantitative point-of-care assay for CRP can be used as a rapid tool for this purpose, in comparison with an automated referral laboratory assay.

3. Methods

3.1 Ethics

The project was approved by the Ethical Review Panel of the University of Nottingham School of Veterinary Medicine and Science.

3.2 Part 1 – Retrospective study

3.2.1 Data collection and processing

Data were collected from the databases of the three CVS Laboratories (Finn, Axiom and Greendale) using specific input and report output terms, detailed in Table 6. The data mining of the database was conducted on the author's behalf by a CVS Labs employee and the resulting data were edited by the same person to remove any identifying or personal information to comply with company policy on data protection and to preserve the anonymity of individual clients and practices involved.

Table 6. Database search terms used to collect retrospective data on canine BAL cases submitted to CVS Laboratories.

Data Search Inputs	Outputs
Lab test code for BAL/respiratory washes Or relevant cytology test codes	Date of submission
Species – Dog	Lab case ID number
	Practice code
	Species
	Breed
	Sex
	Age
	History
	Cytology report
	Microbiology results (if applicable)
	Additional blood test results (if applicable)

After receiving the data, a process of data cleaning was undertaken to format the entries to a standardized presentation in Microsoft Excel. Due to the differing search methods used to collect the cases from each of the 3 laboratories, there were differing numbers of cases yielded as an output from the search. Variable search methods needed to be used because of different test codes and test groups used by different diagnostic labs. For example, one of the labs had a specific BAL code that could be searched, enabling easy categorization of cases and removal of erroneous ones. However, the other two labs had no specific code that could be searched (just a general “cytology” one) so identifying cases using Excel search formulae was needed (detailed in Appendix 5). None of the labs had specific fields or coding “cytologic diagnosis” that could be isolated, and so Excel formulae were also used to extract

the cytologic interpretation from the cytology reports for each case, enabling categorisation by diagnosis. No separate 'Interpretation' or 'Diagnosis' field was available to easily sort cases. The keywords searched for common words often used in the initial section of the cytology report where the type of samples received are stated. This search was very sensitive in retrieving relevant cases, but occasionally included unwanted ones. These were removed manually.

3.2.2 Data analysis

The retrospective data retrieved from the laboratory databases were analysed to further investigate the characteristics of dogs with infectious lower respiratory disease, and other lower airway disease, and the prevalence of those diagnoses.

Data was analysed to investigate the effects of various signalment characteristics, such as age, sex, and breed on cytologic diagnosis. Any differences in cultured microorganisms from cases with different cytologic diagnoses were also analysed. Cases from brachycephalic dogs were also analysed separately to investigate the potential impact of these airway morphology changes on the prevalence and risk of cytologic diagnoses and microbial culture.

The effect of location within the country in terms of a rural versus urban environment was also investigated to determine whether these environmental factors impacted the prevalence of different cytologic diagnoses. This was achieved using the Office for National Statistics (ONS) 2011 postcode classification (Office for National Statistics, 2011). This dataset gives a pre-defined classification of rural or urban for a particular postcode based on

the 2011 census data. Each postcode is coded A1, B1, C1 or C2 for urban postcodes or D1, D2, E1, E2, F1 or F2 for rural postcodes, depending on the density and size of settlement. The postcode of the veterinary practice from which cases were submitted (either primary care or referral) were used to classify cases, as no details of the home addresses of owners are collected by the laboratories.

Statistics were performed using the GraphPad Prism 8 statistics package, Microsoft Excel, and the MedCalc Odds Ratio calculator. Odds ratios were calculated to assess the influence of breed on the likelihood of different cytologic diagnoses. The most prevalent pedigree breeds for each disease outcome (each cytologic diagnosis) were compared against cases with no cytologic abnormalities as the negative disease outcome. Crossbreed dogs were used as the control group for each assessment. 95% confidence intervals were also calculated. The formula for calculating the odds ratios was:

$$Odds\ ratio = \frac{disease\ outcome\ (\frac{exposed}{control})}{no\ cytologic\ abnormalities\ (\frac{exposed}{control})}$$

Data of the total case submissions to the three laboratories over a recent six-month period was obtained to use as a comparison population. This could then enable more robust evaluation of the breed prevalence of BAL cases compared to the general case submission population.

3.3 Part 2 – Prospective CRP study

To investigate the relationship of CRP with cases of respiratory disease we recruited BAL washes for cytology and microbiological culture from cases of lower respiratory disease seen in various first-opinion and referral veterinary practices.

3.3.1 Sample collection and case recruitment

When BAL was clinically indicated and consent obtained, vets submitted wash fluid in both plain and EDTA-preserved tubes for both culture and cytology respectively. Along with airway wash samples, surplus serum from these patients was submitted to allow for CRP analysis. The serum was usually obtained from blood samples already taken for pre-anaesthetic blood tests, which may be analysed in-house at the practice, or sent the day before the intended procedure to the referral diagnostic laboratories. Occasionally serum was obtained from blood samples sent to the referral laboratories at the same time as the cytology samples. It was emphasised to clinicians that the procedure required no deviation from normal clinical practices for these cases, an important element in both uncomplicated case recruitment, and ensuring appropriate sample collection and processing. A summary of the sample collection and workflow through the laboratory is presented in Figure 4.

Once samples were collected, completion of a submission form was required (Appendix 2), after which samples were sent, along with any relevant clinical history, to the practice's usual CVS diagnostic laboratory. The samples were then forwarded within 24 hours to Finn Pathologists for analysis, if previously sent to a different location. If blood tests were

performed at the practice, then a copy of the results were requested, along with the serum sample.

A consent form was required to be completed prior to patients being included in the study, which detailed a summary of the project and its intended aims, as well as information on consent and contact details for the project team. This consent form was kept securely for the duration of the project. Information sheets were also created for practice staff to give to clients giving more details about the project before they are included. A similar document was also made available for practice staff, specifying what samples were required and the process involved.

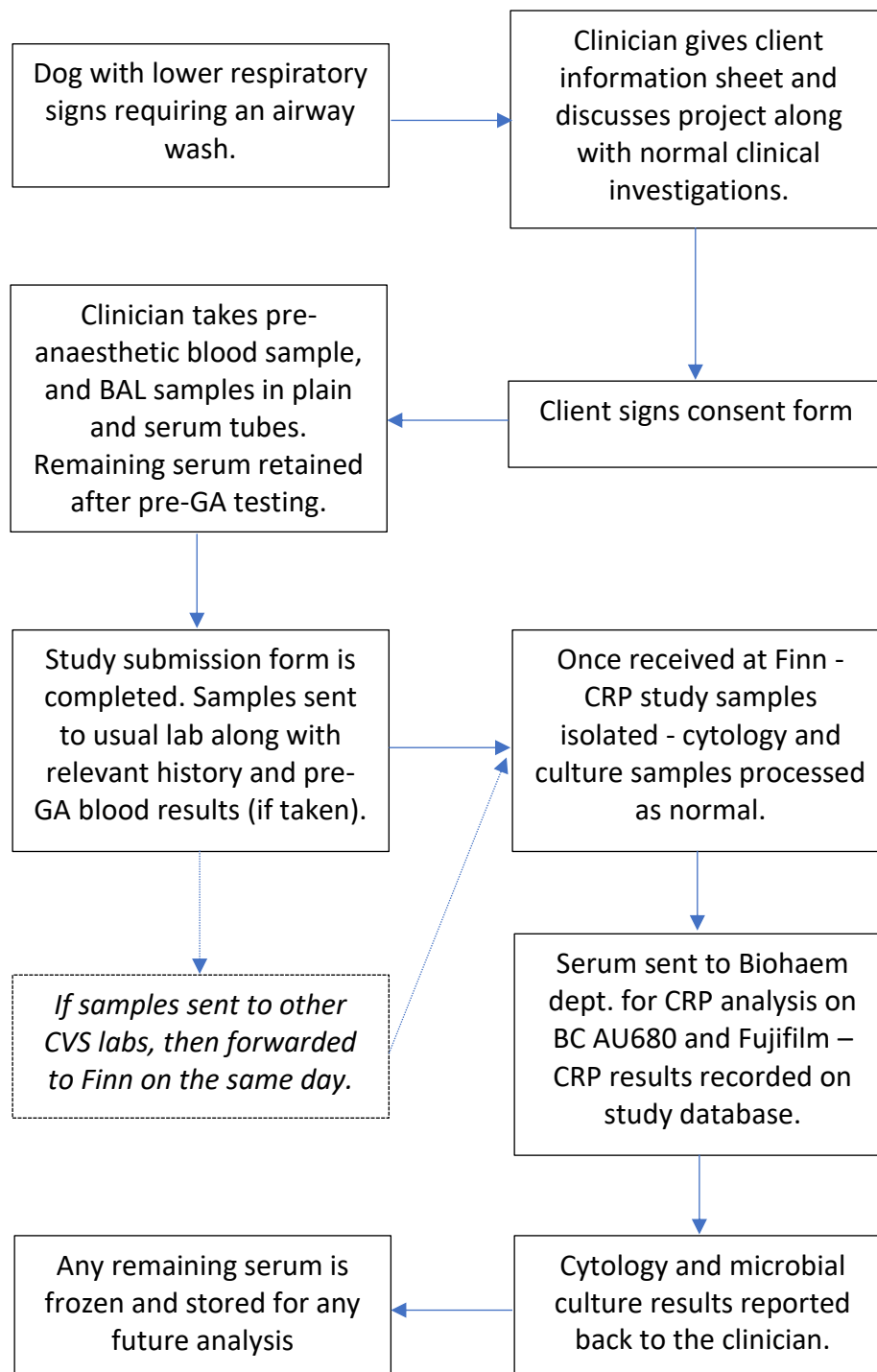


Figure 4 – Flow diagram of sample collection and workflow.

CVS practice staff received various communications via email and the internal intranet system to raise awareness of the project and to give details on what types of cases could be

included, and the samples required. Various email reminders and regular news items in the company literature were subsequently circulated to ensure continued awareness. Alerts and links were also built into the company-wide practice management system to further prompt veterinary surgeons and other practice staff to include cases once they estimate for or billed for an airway wash procedure.

Airway wash cases submitted to the lab without serum samples for the CRP study were noted and the practice submitting the sample was called to request whether they and the owner of the patient would like to take part in the study, and if there was sufficient spare serum remaining from any blood tests performed within 48 hours of the airway wash sample being collected.

Various inclusion and exclusion criteria were created to ensure only relevant cases are examined (Table 7). All dogs of any age, sex, breed or neutered status could be included, and cases needed to be those with lower respiratory tract signs consistent with the need for an airway wash as part of their clinical investigations. This included dyspnoea, abnormal lung sounds, cough, lethargy, and possible pyrexia, among others. Airway samples could be taken blind or with endoscopic guidance, at the discretion of the clinician and availability of practice facilities. Other diagnostic investigations, such as thoracic radiography, echocardiography, or advanced imaging could also be undertaken to investigate cardiorespiratory disease aetiologies; and submitting clinicians were required to provide relevant history and findings to enable complete analysis and interpretation of the samples submitted.

Table 7. Inclusion and exclusion criteria for prospective case recruitment.

Cases for Inclusion	Cases to be Excluded
<ul style="list-style-type: none">• Dogs of any age/breed/sex• BAL samples• Tracheal wash samples (may be analysed separately if enough received)• Cases that have EDTA and plain sample received for cytology and microbial culture respectively.• Cases with blood sample taken <48h prior to airway wash – needs to be minimum of pre-GA bloods either in-house or referral. – If in-house then results need to be sent from the practice.• Relevant clinical history and a consent form received.	<ul style="list-style-type: none">• Cases with previously diagnosed concurrent infectious, inflammatory or neoplastic diseases (which may affect CRP result).• Cases with any concurrent injuries/wounds, or those who have had recent surgery.• Cases where a blood sample has been taken >48 hours prior to airway wash.• Cases with cytology comprising >1% squamous epithelial cells, or cases with Simonsiella present – indicative of oropharyngeal contamination.

3.3.2 Sample processing

Once received, the samples were processed at one laboratory, and cytology performed by the two available on-site ACVP board-certified clinical pathologists. Cytology and microbiological culture results (and any other standard tests requested by the clinician) were reported as normal via the practice's normal diagnostic laboratory. CRP results however, were not made available to the clinician at that point, but a summary of the project findings will be available and sent to any submitting practices, along with appropriate recommendations and guidelines for future use in suitable cases if feasible.

Analysis of CRP was performed on submitted serum on the same day the sample was received at Finn Pathologists to ensure minimal sample degradation or variability in sample

quality. Samples have been shown to be stable at -80°C for 3 months and for 14 days at 4°C or room temperature (Hillström *et al.*, 2015). Samples were analysed on the Beckman Coulter (BC) AU680 automated clinical chemistry machine first with the result recorded in the project spreadsheet available to laboratory staff. Any samples received on a particular day were analysed in one batch. The samples were then run on the Fujifilm DRI-CHEM NX500 with the result recorded in a similar manner. The Fujifilm machine is not completely automated, so samples needed to be run individually.

After analysis serum samples were stored in a -80°C freezer until destruction on completion of the study. Data was stored securely on password protected hardware for the duration of the project. Consent for data collection, storage, and use, including for publication was obtained from clients prior to their inclusion.

3.3.3 CRP assays

The samples were analysed using both the point-of-care Fujifilm Dri-Chem vc-CRP-P assay (Fujifilm Corporation, Tokyo, Japan) on the Fujifilm Dri-Chem NX500 benchtop analyser (Fujifilm Corporation), and the Gentian cCRP turbidometric immunoassay (Gentian Diagnostics AS, Moss, Norway) on the Beckman Coulter AU680 Biochemistry analyser (Beckman Coulter, Brea, CA, USA) as the reference assay. The Fujifilm cCRP assay is a canine-specific quantitative dry chemistry assay containing a monoclonal mouse anti-canine CRP antibody. The Gentian cCRP assay is an immunoturbidometric method utilizing a polyclonal chicken anti-canine antibody, which is intended for use with larger automated clinical chemistry analysers.

The manufacturer reported analytical ranges were 3-70 mg/L for the Fujifilm cCRP and 10-300 mg/L for the Gentian cCRP assay. The imprecision (2.4%) and limit of quantification (LoQ) (6.8 mg/L) for the Gentian cCRP assay have been previously published by other authors (Hillström *et al.*, 2014). Any results below the LoQ are reported as < 6.8 mg/L, and therefore for the purposes of statistical analysis for this study any values < 6.8 were designated as 50% of this value (3.4 mg/L). Similarly, the minimum reportable value for the Fujifilm cCRP assay is 3 mg/L, therefore any results < 3 mg/L were set at 1.5 mg/L. Any samples above the linear range (>70 mg/L for the Fujifilm cCRP assay, and >300 mg/L for the Gentian cCRP assay) were diluted and an absolute value reported after calculation.

3.3.4 Disease definitions

Cases were classified into seven specific disease groups and a category for cases with no abnormalities after analysis of cytology and microbiological culture. These groups were: Septic neutrophilic inflammation, neutrophilic inflammation, eosinophilic inflammation, macrophagic inflammation, mixed inflammation, chronic irritation or hyperplasia, and other causes, such as neoplasia or haemorrhage. Criteria for categorisation are given in Table 8. These criteria were discussed and agreed with the clinical pathologists that viewed and reported the cases.

Table 8. Criteria for disease categorisation based on airway wash cytology and microbial culture.

Disease group	Criteria
Septic neutrophilic inflammation	Cytologic confirmation of intracellular bacteria within neutrophils, usually with increased percentage of neutrophils (>5%).
Neutrophilic inflammation	>5% neutrophils on total nucleated cell count, with no evidence of infection (i.e. no intracellular bacteria).
Eosinophilic inflammation	>5% eosinophils on total nucleated cell count.
Macrophagic inflammation	Increase in total cellularity with predominance of macrophages – macrophages are frequently vacuolated or multinucleated.
Mixed inflammation	Mix of leukocytes (neutrophils, eosinophils or macrophages) with increased cellularity.
Epithelial hyperplasia	Goblet cell hyperplasia, increased mucus.
Other	Absence of findings consistent with inflammatory or infectious disease as above, but presence of other abnormalities such as neoplastic cells or pulmonary haemorrhage. <i>(If prior disease known before recruitment into study, then the case will be excluded).</i>
No abnormalities detected (NAD)	Normal cytologic findings – predominantly macrophages, epithelial cells. Low overall cellularity.

3.3.5 Data analysis

Once data collection was completed, the data were analysed to determine if there were any statistical differences between cases diagnosed with septic neutrophilic inflammation and non-septic cases. Analysis was also completed to determine if there were any significant differences between the two analytical methods used in the study (Fujifilm cCRP assay and Gentian cCRP assay). One-way ANOVA tests with post-hoc comparisons were performed using GraphPad Prism 8.

Analysis was conducted to assess the diagnostic validity of the tests and their ability to discriminate between cases with or without infection. These evaluations included sensitivity, specificity, negative and positive predictive values, and receiver operator characteristic (ROC) curves. These results then allowed assessment of clinical utility and determination of proposed clinical decision limits.

3.4 Part 3 – Method comparison study

The aim of the method comparison study was to estimate the type of error and magnitude of error between these two methods, to assess the inherent imprecision of the point-of-care assay, and to be able to judge the acceptability of the assay and reproducibility of results within defined quality specifications and performance goals (Jensen and Kjølgaard-Hansen, 2006).

Method comparison studies require definition of acceptable analytical error of a method and the acceptable difference between two assays. The amount of analytical error that is allowed without compromising patient care or result interpretation can be defined from a variety of sources. The most widely used are acceptance limits based on analytical quality specifications, which can be derived from biologic variation or consensus opinions from clinicians (Flatland *et al.*, 2018). Quality goals for this study were defined from a previous study of CRP biologic variation (Kjølgaard-Hansen *et al.*, 2003), and included in the ASVCP

total error guidelines for biochemistry (Harr *et al.*, 2013). Desirable total allowable error (desTE_a) was defined as 29.6%, minimum total allowable error (minTE_a) was defined as 44.4%, and allowable imprecision as 12.2% (Harr *et al.*, 2013). Assessment of imprecision and bias would enable the calculation of observed error of the assay (TE_{obs}), which could then be compared with the TE_a to judge acceptability of the assay.

For the purpose of this study serum samples were analysed using the point-of-care Fujifilm cCRP assay on the Fujifilm Dri-Chem NX500 benchtop analyser, and compared with the Gentian cCRP turbidometric immunoassay on the Beckman Coulter AU680 Biochemistry analyser as a reference assay, as described above in section 3.3.3.

3.4.1 Sample collection

Samples for the method comparison study were initially collected on a random basis from canine serum samples sent to the laboratory for other testing. Cases were selected regardless of breed, age or sex. Surplus serum was utilized from completed biochemistry testing. However, to obtain adequate samples with high CRP values, further cases were selected based on observation of neutrophilia with left shift on haematology results or blood smear review, or based on an appropriate clinical history of infectious or inflammatory disease. Fifty samples were acquired for the study using this method. This yielded a higher proportion of high CRP results compared with random sampling although it was not guaranteed to give elevated results with each case. It is recommended that at least 40 patient samples are required for a method comparison study according to published method comparison guidelines (Jensen and Kjelgaard-Hansen, 2006; Harr *et al.*, 2013). Samples were stored at -20°C until analysis.

3.4.2 Sample Analysis

Method comparison guidelines suggest the assessment of linearity and imprecision of the new method before comparison with the standard method (Westgard, 2008). For the imprecision study, the samples were run in triplicate over two days and refrigerated at 4°C between runs. Two serum pools of expected Low and high concentration were created for this purpose. The samples for the linearity study were run in duplicate over two different days from a pooled serum sample at a concentration of 64 mg/L. They were diluted using the Fujifilm CRP diluent used to run the test to ensure similarity to the processing of clinical samples. Dilutions of 0.5, 0.25, 0.125 and 0.0625 of the original concentration were analysed. Samples used for method comparison were run in batches of 5-10, and each batch was analysed by both machines on the same day to avoid differences due to instability. For expected high CRP samples collected later in the study timeline, the samples were run on both methods as they were received. Calibration of the methods was performed before each run using the manufacturer provided calibration card for the Fujifilm assay, and using the Gentian Canine CRP calibrator kit for the Gentian assay. Two levels of quality control material (low and high) were analysed once daily for the Gentian cCRP assay using the manufacturer control kit. The Fujifilm analyser automatically diluted the sample 21 times prior to analysis using the manufacturer provided diluent. Further details about the manufacturer reported analytical ranges, previously reported imprecision and LoQ for the Gentian cCRP assay, and statistical calculations to analyse results outside of the linear ranges are detailed in the previous section – 3.3.3. *CRP assays*.

3.4.3 Data Analysis

Statistical analysis was performed using Microsoft Excel and GraphPad Prism Version 8. (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at $p = 0.05$, and data was initially analysed for normality using a Shapiro-Wilk test.

Imprecision was evaluated by calculating a coefficient of variation (CV) from the mean and standard deviation of the data [$CV\% = SD/\text{mean}$] using formulae for either replicate (intra-assay) or multiple duplicate (inter-assay) analysis. Linearity of the Fujifilm cCRP assay under dilution was evaluated by plotting observed against expected values with slope and intercept calculation by ordinary least squares regression analysis. Results from the method comparison study were initially assessed for normality and subsequent correlations were then assessed with a Pearson or Spearman method for parametric or non-parametric data respectively. The appropriate regression analysis was then selected based on the calculated r values as per published recommendations (Jensen and Kjelgaard-Hansen, 2006; Arnold *et al.*, 2019). Accordingly, simple linear regression was used when r was >0.99 for data with a wide range, or >0.975 for data with a narrow range. When r was below these values, a Deming regression was selected, which allowed for the inherent relative uncertainty in both methods using calculated SD variables. The SD of the Fujifilm cCRP assay was taken from the precision study, whereas the SD of the Gentian cCRP assay was taken from the previous 30 days of quality control data from use of the assay in the laboratory on the Beckman Coulter AU680. Bland-Altman difference plots for both absolute and percentage difference were constructed to assess agreement between the two methods. This enabled calculation of the average bias of the methods with 95% confidence intervals.

Analytical performance was compared to the defined quality specifications stated above.

Total observed error (TE_{obs}) was calculated with the formula: TE_{obs} = bias% + (2 x CV%).

This could then be compared with both desirable and minimum TE_a to assess assay performance. A method is deemed acceptable for clinical use if TE_{obs} < TE_a. Sigma (σ) metrics were also calculated to provide a quantified assessment of overall analytical performance within the defined TE_a quality specifications. Sigma values were calculated using the following formula:

$$s = \frac{[TE_a (\%) - \text{bias} (\%)]}{CV (\%)}$$

The calculated σ values were interpreted using the following criteria: <2 = unacceptable, >2 = Poor, >3 = marginal, >4 = good, >5 = excellent, >6 = world class (Nevalainen *et al.*, 2000; Westgard, 2006). Generally any assay performing at the 3-sigma level is considered the minimum acceptable performance for a clinical analytical process (Westgard, 2006). Medical Decision (MEDx) charts were also constructed using a downloaded calculator provided by Westgard QC (<https://www.westgard.com/downloads/worksheets-downloads.html>) in conjunction with Microsoft Excel. MEDx charts provide a graphical representation of assay performance by assessing inaccuracy and imprecision relative to a defined quality requirement, and have previously been incorporated in veterinary studies (Westgard, 1995; Jensen, Iversen and Høier, 1999).

4. Results

4.1 Part 1 – Retrospective Epidemiological study

4.1.1 Sample population

Database searches involved extracting relevant information related to recent cytology case submissions, as detailed in Table 9. Different volumes of cases were collected from the different labs due to varying amounts of time that the labs have been using the system. Data from the past 4 years, up to July 2018 were collected from Axiom and Finn, and from the past 1 year from Greendale. In total 2501 BAL cases were retrieved for analysis. 1735 cases or (69.4%) had a microbial culture request submitted along with the cytology sample.

4.1.2 Cytological Findings

Initial analysis of different cytologic interpretations was conducted using a similar keyword search approach but focused on the part of the text report including the cytologic interpretation of the case which was then extracted from the report and searched for keyword phrases based on different differentials. To enable investigation of the likely usefulness of CRP in relevant cases of infectious aetiology, it was important to establish the frequency of a cytological diagnosis of sepsis in these patients. A total of 405 cases out of 2501 (16.2%) had an interpretation of bacterial sepsis, although the prevalence amongst cases with “diagnostic” cytologic findings (after removing “non-diagnostic” cases) was 17.9%.

Table 9. Summary of cases collected from retrospective database search. Numbers of case entries and initial processed cases are shown with key findings of sepsis cases.

Total number of entries	47,296	
Total number of cases (after removal of duplicates and erroneous entries)	40,518	
Number of BAL cases	2,501	
Number of BAL cases with microbial culture	1,735	69.4% (% of total BAL cases)
Cases with a diagnosis of sepsis	405	16.2% (% of total BAL cases) 17.9% (% of diagnostic BAL cases, 2260 cases)
Non-diagnostic cases	241	9.6% (% of total BAL cases)
Cases with sepsis and microbial culture submitted	325	80.2% (% of sepsis cases)
Cases with sepsis and positive microbial culture	237	58.5% (% of sepsis cases) (% of sepsis cases)

All cases categorised by cytologic diagnosis are summarized in Table 10. Mixed inflammation was the most commonly diagnosed abnormality (25.1%), and 17.7% of cases had no cytologic abnormalities detected (NAD). In total, 241 cases (9.6%) were cytologically non-diagnostic cases or cases with significant oropharyngeal contamination.

Table 10. Frequency of cases in each category of cytologic diagnosis with percentages of total BAL cases for each category in brackets.

Cytologic Diagnosis	Frequency
No Abnormalities Detected (NAD)	443 (17.7%)
Mixed	629 (25.1%)
Neutrophilic	428 (17.1%)
Sepsis	405 (16.2%)
Macrophagic	138 (5.5%)
Eosinophilic	115 (4.6%)
Hyperplasia	57 (2.3%)
Other	45 (1.8%)
<i>.Non-diagnostic</i>	241 (9.6%)

4.1.3 Microbiological Findings

Microbial culture was performed in 1735 of 2501 cases (69.4%). Sepsis was diagnosed by cytology in 405 cases, 325 of which had microbial culture performed, with sepsis confirmed with a positive microbial culture in 237 (73%) of those cases, as shown in Table 11. In addition, 87 (27%) cases with a cytologic diagnosis of sepsis had no organisms cultured. Conversely, 33% of cases with no cytologic abnormalities had a positive microbial culture. Cases diagnosed with non-septic cytologic abnormalities had a positive microbial culture in 26-43% of cases, but the majority of cases in all groups except for sepsis cases had no organisms isolated on culture.

Table 11. Percentage and frequency of cases with microbial culture requested that yielded no organisms or a positive culture result for each category of cytologic diagnosis.

Cytologic Diagnosis	No organisms cultured	Organisms isolated
NAD	67%	33%
	190	92
Sepsis	27%	73%
	87	237
Neutrophilic Inflammation	64%	36%
	187	104
Macrophagic Inflammation	74%	26%
	65	23
Eosinophilic Inflammation	70%	30%
	64	27
Mixed Inflammation	57%	43%
	269	199
Hyperplasia	63%	37%
	22	13
Other	70%	30%
	21	9

The most frequent bacterial isolate result in each group was a mixed infection. A mixed infection was cultured in 49% of sepsis cases, 29% of cases diagnosed with neutrophilic inflammation without sepsis, and 25% of NAD cases with a positive microbial culture. The most frequently cultured single isolates in sepsis cases were *Bordetella bronchiseptica* (12% of sepsis cases), *Pasteurella* spp. (10%), *E. coli* (7%), Lactose-fermenting coliforms (6%), unclassified coliforms (5%), *Pseudomonas* spp. (5%), and coagulase-positive *Staphylococcus* spp. (1%). The most frequent single organisms isolated from cases diagnosed with neutrophilic inflammation were *Pasteurella* spp. (18%), *E. coli* (12%), and *B. bronchiseptica* (11%). *Pasteurella* spp. were also the most frequently isolated organisms in cases with no cytologic abnormalities (13% of cases), with unclassified coliforms and *E. coli* also isolated. Similar organisms were also isolated in other groups, with variable prevalence.

4.1.4 Breed Analysis

Analysis of breed prevalence (Table 12 and Figure 5) showed that crossbreeds were the most prevalent breed amongst canine BAL submissions (396 cases), followed by Labrador retrievers (276 cases), Jack Russell Terriers (190 cases), English Springer Spaniels (127 cases), West Highland White Terriers (106 cases) and Staffordshire Bull Terrier (95 cases) as the five most common pedigree dogs. The most frequent brachycephalic breeds submitted were Cavalier King Charles Spaniels, Bulldogs, Shih-Tzus, Boxers and Pugs. A total of 229 cases were from breeds associated with brachycephalic conformation, of which 195 were cytologically diagnostic.

There were significantly higher percentages of BAL cases compared with total laboratory submissions in 8 of the top 21 breeds, when analysed with a Chi-square test against all other breeds from the total submissions. These breeds were Jack Russell Terriers, English Springer Spaniels, West Highland White Terriers, Cavalier King Charles Spaniels, English Cocker Spaniels, Siberian Huskies, Bulldogs, and Whippets. Five breeds had significantly lower percentages of BAL cases compared with expected proportions. These breeds were crossbreed dogs, Cocker Spaniels, Golden Retrievers, Boxers, and German Shepherd dogs.

Table 12. Summary table showing the number of cases of different breeds for submitted BAL cases and the percentage of total cases submitted to the laboratory for those breeds for comparison. The top twenty purebred breeds are shown in addition to the number of crossbreed dogs. Significance of observed versus expected percentages were measured with a Chi-square test. *** - $P < 0.001$, * - $P < 0.05$. Breeds highlighted in green have a lower percentage of BAL cases compared with the total laboratory caseload. Breeds highlighted in orange were overrepresented in the BAL population compared to the laboratory caseload.

Breed	BAL Cases	% BAL Cases	% Laboratory Caseload	Significant difference	
Crossbreed	396	15.83%	18.24%	$P < 0.001$	***
Labrador Retriever	276	11.04%	10.73%	$P = 0.112$	
Jack Russell Terrier	190	7.60%	4.03%	$P < 0.001$	***
English Springer Spaniel	127	5.08%	2.20%	$P < 0.001$	***
West Highland White Terrier	106	4.24%	2.47%	$P < 0.001$	***
Staffordshire Bull Terrier	95	3.80%	4.12%	$P = 0.079$	
Border Collie	85	3.40%	2.71%	$P = 0.322$	
Cocker Spaniel	71	2.84%	4.25%	$P < 0.001$	***
Cavalier King Charles Spaniel	65	2.60%	1.80%	$P = 0.044$	*
Yorkshire Terrier	60	2.40%	1.71%	$P = 0.080$	
English Cocker Spaniel	52	2.08%	1.17%	$P = 0.024$	*
Siberian Husky	43	1.72%	0.56%	$P < 0.001$	***
Border Terrier	42	1.68%	1.83%	$P = 0.191$	
Bulldog	35	1.40%	0.58%	$P = 0.001$	***
Whippet	35	1.40%	0.53%	$P < 0.001$	***
Golden Retriever	35	1.40%	2.67%	$P < 0.001$	***
Bichon - Frise	34	1.36%	1.45%	$P = 0.293$	
Lurcher	31	1.24%	0.92%	$P = 0.811$	
Boxer	29	1.16%	1.93%	$P < 0.001$	***
Beagle	28	1.12%	1.05%	$P = 0.759$	
German Shepherd Dog	27	1.08%	2.28%	$P < 0.001$	***

Breed prevalence was assessed for each cytologic diagnosis (as shown in Table 14).

Crossbreeds were the most prevalent breed for each category apart from the Other category. Prevalence of crossbreeds ranged from 13% to 23% of cases in these groups. The second most prevalent breed in each group was either Jack Russell Terriers, Labrador Retrievers, or in the case of septic neutrophilic inflammation, English Springer Spaniels. The former two breeds are the two most prevalent breeds of all submissions to the laboratories.

Further analysis of the influence of breed on the likelihood of different cytologic diagnoses was assessed by calculating odds ratios. A comparison was made of the top pedigree breeds for each disease outcome (cytologic diagnosis) against cases with no cytologic abnormalities as the negative disease outcome (Table 15). Crossbreed dogs were used as the control population as they were the most frequent breed in each group. Various breeds showed a significant association with a particular diagnosis. English Springer Spaniels and Yorkshire Terriers show a significantly increased odds of a diagnosis of septic neutrophilic inflammation (Odds ratio [OR] 1.986, $p = 0.03$ and OR 3.832, $p = 0.01$ respectively). There was also a significant relationship between Yorkshire Terriers and a diagnosis of macrophagic inflammation (OR 6.311, $p = 0.003$), and although not statistically significant (with significance set at $p < 0.05$), there appeared to be an increased probability of a diagnosis of sterile neutrophilic inflammation in this breed (OR 2.56, $p = 0.09$). In contrast, Border Collies showed decreased odds of a diagnosis of both neutrophilic (OR 0.437, $p = 0.03$) and mixed inflammation (OR 0.387, $p = 0.007$). Mixed inflammation is often composed of a neutrophilic infiltrate combined with a further cell type, which may explain the relationship. The Siberian Husky appeared to be at increased risk of both eosinophilic (OR 7.474, $p = 0.003$) and mixed inflammation (OR 3.302, $p = 0.04$). Similar to the situation with

neutrophilic inflammation, a mixed cell inflammation frequently contains eosinophils.

Whippets also showed a significantly increased probability of being diagnosed with eosinophilic inflammation (OR 6.455, $p = 0.005$).

4.1.4.1 Brachycephalic dogs

Analysis of the impact of brachycephalic conformation on the prevalence of different cytologic diagnoses from BAL samples was also performed which showed no significant differences compared with the total population, however there were a higher percentage of cases with septic neutrophilic inflammation, eosinophilic inflammation, macrophagic inflammation, and hyperplasia (Table 13). There was also a lower percentage of cases with mixed inflammation, neutrophilic inflammation, and cases without any cytologic abnormalities compared with the total caseload.

A further assessment of any differences in microbial culture from brachycephalic dogs showed that out of the 138 cases with microbial culture requested, approximately 50% yielded microorganisms. A mixed isolate was cultured most frequently, with 21% of cases yielding this result. *B. bronchiseptica* was the most frequently isolated single organism (11%), followed by lactose-fermenting coliforms (6%), and *Pasteurella* spp. (3%). Of those cases with septic neutrophilic inflammation 76% showed a positive culture, and mixed isolates accounted for 38% of cases, with *B. bronchiseptica* cultured in 24% of cases. No organisms were found in 24% of cases. These are similar findings to the caseload as a whole, however the percentage of cases with *B. bronchiseptica* was higher for all cases (11% vs 5%) and for septic inflammation cases (24% vs 12%).

Table 13. Frequency of brachycephalic breed cases in each category of cytologic diagnosis, with the percentage of diagnostic brachycephalic cases (195 brachycephalic breed cases in total), in comparison to the total BAL caseload. Significance of observed versus expected percentages were measured with a Chi-square test. $P < 0.05$ is considered significant. No results showed statistical significance at this level.

Cytologic diagnosis	Brachycephalic cases	% diagnostic brachycephalic cases	% Total caseload	Significance
NAD	32	16.4%	17.7%	$P = 0.852$
Sepsis	43	22.0%	16.2%	$P = 0.334$
Neutrophilic	33	16.9%	17.1%	$P = 0.977$
Eosinophilic	20	10.3%	4.6%	$P = 0.210$
Macrophagic	17	8.7%	5.5%	$P = 0.595$
Mixed	35	17.9%	25.1%	$P = 0.340$
Hyperplasia	10	5.1%	2.3%	$P = 0.615$
Other	5	2.6%	1.8% ¹	$P = 0.906$

4.1.5 Sex and neuter status

Of the BAL cases collected, 382 were entire females, 724 were neutered females, 561 were entire males, and 738 were neutered males. 96 had no sex or neutering status recorded. No significant differences between male and female cases or neutered or entire cases were found with a Chi-square test.

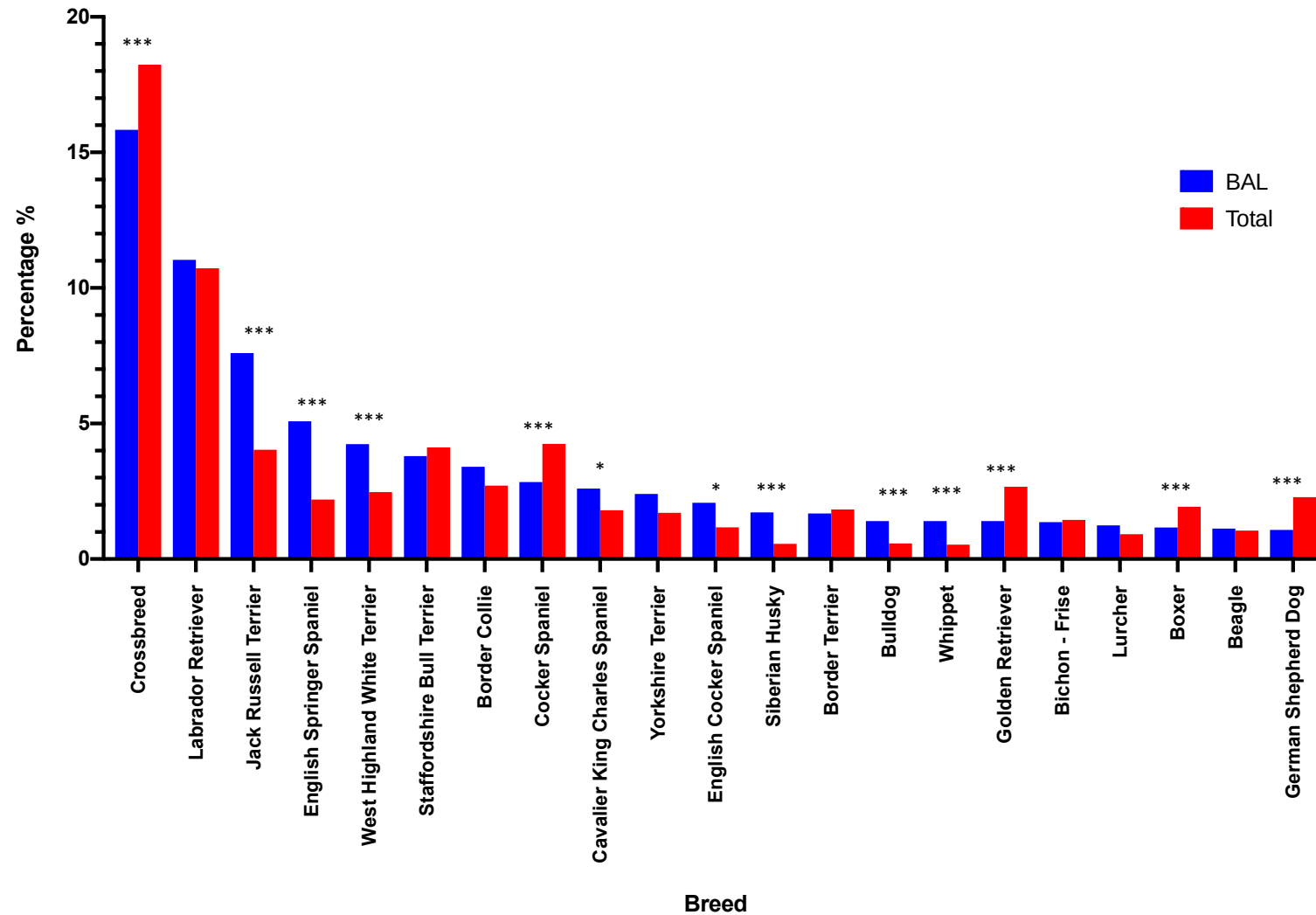


Figure 5 – Percentages of BAL cases from the most frequently submitted 20 pedigree breeds and crossbreed dogs, compared with the percentages of cases from total submissions to the laboratories. Significance of observed versus expected percentages were measured with a Chi-square test. *** - $P < 0.001$, * - $P < 0.05$.

Table 14. Prevalence of breeds seen in each category of cytologic diagnosis. The 10 most prevalent breeds are displayed for each category, with the exception of Hyperplasia, where only the top 7 breeds had more than one case. The percentages are the percentage of the total number of cases with that particular cytologic diagnosis.

NAD		Septic		Neutrophilic		Eosinophilic	
Crossbreed	16%	Crossbreed	14%	Crossbreed	14%	Crossbreed	19%
Labrador Retriever	12%	English Springer Spaniel	8%	Labrador Retriever	13%	Jack Russell Terrier	15%
Border Collie	7%	Labrador Retriever	8%	Jack Russell Terrier	7%	Siberian Husky	10%
Jack Russell Terrier	7%	West Highland White Terrier	5%	Staffordshire Bull Terrier	5%	Labrador Retriever	8%
English Springer Spaniel	5%	Jack Russell Terrier	4%	English Springer Spaniel	5%	Whippet	7%
Staffordshire Bull Terrier	4%	Bulldog	4%	West Highland White Terrier	5%	Border Terrier	3%
West Highland White Terrier	3%	Yorkshire Terrier	4%	Cocker Spaniel	5%	Cavalier King Charles Spaniel	3%
Cocker Spaniel	3%	Cocker Spaniel	3%	Cavalier King Charles Spaniel	3%	Golden Retriever	3%
Cavalier King Charles Spaniel	3%	Staffordshire Bull Terrier	2%	Border Collie	3%	Staffordshire Bull Terrier	2%
Bichon-Frise	3%	Border Terrier	2%	Yorkshire Terrier	3%	Flat Coated Retriever	2%

Mixed		Macrophagic		Hyperplasia		Other	
Crossbreed	17%	Crossbreed	13%	Crossbreed	23%	Labrador Retriever	16%
Labrador Retriever	10%	Labrador Retriever	7%	Jack Russell Terrier	7%	Jack Russell Terrier	11%
Jack Russell Terrier	8%	Yorkshire Terrier	6%	Lhasa Apso	5%	Golden Retriever	9%
Staffordshire Bull Terrier	5%	Jack Russell Terrier	5%	Labrador Retriever	4%	German Shepherd Dog	7%
English Springer Spaniel	5%	Cavalier King Charles Spaniel	4%	Golden Retriever	4%	Cocker Spaniel	5%
West Highland White Terrier	5%	West Highland White Terrier	4%	Border Terrier	4%	Border Terrier	5%
Siberian Husky	3%	English Springer Spaniel	3%	Unknown	4%	Crossbreed	5%
Cavalier King Charles Spaniel	3%	Staffordshire Bull Terrier	3%			Staffordshire Bull Terrier	5%
Border Collie	3%	Border Collie	3%			West Highland White Terrier	5%
Border Terrier	3%	Cocker Spaniel	2%			Shih-tzu	5%

Table 15. Odds ratios for the top pedigree breeds found in each category of cytologic diagnosis. The odds of each cytologic diagnosis are compared against the odds of a negative disease outcome (no cytologic abnormalities). Crossbreed dogs are used as the control group. Data shaded in green is statistically significant ($p < 0.05$).

Disease outcome	Test group	Odds ratio	95 % CI:	Significance level
Septic	English Springer Spaniel	1.9856	1.0534 to 3.7429	P = 0.0339
	Labrador Retriever	0.7585	0.4392 to 1.3102	P = 0.3217
	West Highland White Terrier	1.6529	0.7896 to 3.4602	P = 0.1825
	Jack Russell Terrier	0.7772	0.4005 to 1.5084	P = 0.4563
	Bulldog	No normal cases		
	Yorkshire Terrier	3.8317	1.3365 to 10.9857	P = 0.0124
	Cocker Spaniel	1.0465	0.4574 to 2.3943	P = 0.9143
	Staffordshire Bull Terrier	0.6525	0.2884 to 1.4761	P = 0.3053
	Border Terrier	2.5357	0.7444 to 8.6380	P = 0.1368
Disease outcome	Test group	Odds ratio	95 % CI:	Significance level
Neutrophilic	Labrador Retriever	1.2311	0.7387 to 2.0515	P = 0.4249
	Jack Russell Terrier	1.2442	0.6753 to 2.2925	P = 0.4835
	Staffordshire Bull Terrier	1.409	0.7015 to 2.8298	P = 0.3352
	English Springer Spaniel	1.2194	0.6123 to 2.4285	P = 0.5726
	West Highland White Terrier	1.7071	0.8144 to 3.5785	P = 0.1567
	Cocker Spaniel	1.6628	0.7746 to 3.5694	P = 0.1920
	Cavalier King Charles Spaniel	1.0744	0.4565 to 2.5288	P = 0.8695
	Border Collie	0.4365	0.2069 to 0.9208	P = 0.0295
	Yorkshire Terrier	2.5607	0.8429 to 7.7788	P = 0.0972
Disease outcome	Test group	Odds ratio	95 % CI:	Significance level
Eosinophilic	Jack Russell Terrier	1.9328	0.8658 to 4.3147	P = 0.1077
	Siberian Husky	7.4737	2.0314 to 27.4959	P = 0.0025
	Labrador Retriever	0.5749	0.2337 to 1.4143	P = 0.2281
	Whippet	6.4545	1.7732 to 23.4955	P = 0.0047
	Border Terrier	2.4205	0.5028 to 11.6519	P = 0.2703
	Cavalier King Charles Spaniel	0.7448	0.1943 to 2.8540	P = 0.6672
	Golden Retriever	2.4205	0.5028 to 11.6519	P = 0.2703
	Staffordshire Bull Terrier	0.3397	0.0733 to 1.5744	P = 0.1676
	Flat Coated Retriever	2.1515	0.3376 to 13.7112	P = 0.4175
Disease outcome	Test group	Odds ratio	95 % CI:	Significance level
Mixed	Labrador Retriever	0.8097	0.4921 to 1.3323	P = 0.4061
	Jack Russell Terrier	1.2241	0.6949 to 2.1564	P = 0.4839
	Staffordshire Bull Terrier	1.2166	0.6276 to 2.3585	P = 0.5615
	English Springer Spaniel	0.9828	0.5081 to 1.9013	P = 0.9590
	West Highland White Terrier	1.3209	0.6445 to 2.7073	P = 0.4471
	Siberian Husky	3.3023	1.0563 to 10.3241	P = 0.0400
	Cavalier King Charles Spaniel	0.9526	0.4253 to 2.1338	P = 0.9060
	Border Collie	0.387	0.1943 to 0.7709	P = 0.0069
	Border Terrier	2.4767	0.7653 to 8.0156	P = 0.1301
Disease outcome	Test group	Odds ratio	95 % CI:	Significance level
Macrophagic	Labrador Retriever	0.6827	0.2842 to 1.6402	P = 0.3933
	Yorkshire Terrier	6.3111	1.8423 to 21.6194	P = 0.0034
	Jack Russell Terrier	0.9521	0.3595 to 2.5218	P = 0.9213
	Cavalier King Charles Spaniel	1.5171	0.4785 to 4.8097	P = 0.4789
	West Highland White Terrier	1.3148	0.4219 to 4.0973	P = 0.6370
	English Springer Spaniel	0.7513	0.2291 to 2.4642	P = 0.6371
	Staffordshire Bull Terrier	0.8304	0.2511 to 2.7457	P = 0.7607
	Border Collie	0.4931	0.1544 to 1.5744	P = 0.2326
	Cocker Spaniel	0.8452	0.2191 to 3.2604	P = 0.8071

4.1.6 Age Analysis

Cases ranged in age from 2 months to 17 years and 4 months. There were 111 cases with no reported age. The data was not normally distributed and so a non-parametric Mann-Whitney test was performed to assess statistical significance. Cases with no cytologic abnormalities had a median age of 8 years 11 months (Range - 2 months to 17 years and 1 month). Septic neutrophilic inflammation cases had a median age of 7 years 11 months (Range – 2 months to 15 years 11 months), significantly lower than NAD cases ($P = 0.015$). Eosinophilic inflammation cases had a significantly lower median age of 4 years 11 months (Range – 2 months to 14 years 3 months) ($P = < 0.001$). No significant difference was found between the median age of each of the other cytologic diagnoses and normal cases.

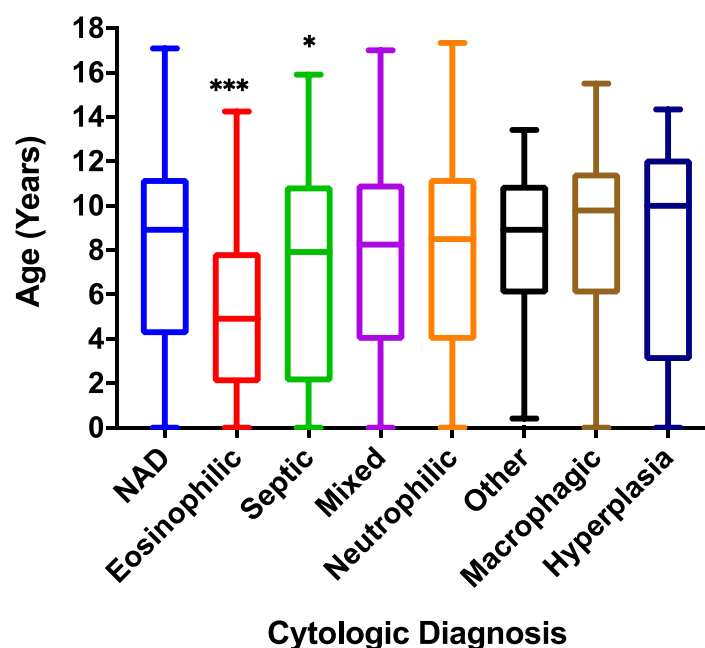


Figure 6. Ages of cases by cytologic diagnosis. The median age is shown with the 25% and 75% percentiles as the box with the whiskers representing the minimum and maximum range. Significance between normal cases (NAD) and other cases measured with a Mann-Whitney test. *** - $P < 0.001$, * - $P = < 0.05$.

4.1.7 Analysis of cases by location

Classification of cases by rural versus urban submission locations showed no significant differences in observed and expected results when measured with a Chi-square test and Fisher's exact test. There were 400 cases (17.7% of diagnostic cases) from a rural location, with the majority of cases, 1860 (82.3%) submitted from an urban location as shown in Table 16.

Table 16. Summary table showing the number and percentage of cases from a rural or urban environment by cytologic diagnosis.

Cytologic Diagnosis	Rural	% Rural	Urban	% Urban
No Abnormalities	82	18.5	361	81.5
Sepsis	73	18.0	332	82.0
Neutrophilic Inflammation	94	22.0	334	78.0
Eosinophilic Inflammation	21	18.3	94	81.7
Macrophagic Inflammation	21	15.2	117	84.8
Mixed Inflammation	98	15.6	531	84.4
Hyperplasia	8	14.0	49	86.0
Other	3	6.7	42	93.3
Total	400	17.7	1860	82.3

4.2 Part 2 – Prospective CRP study

4.2.1 Sample population

A total of 24 prospective cases were included in the study, submitted via Finn Pathologists and Axiom Laboratories from October 2018 to December 2019 (summarized in Table 17). Two cases were excluded due to concurrent non-respiratory disease. One case was diagnosed with acute pancreatitis (confirmed with increased serum lipase, amylase and canine specific pancreatic lipase results), and the other was diagnosed with cardiac disease and pericardial effusion (not included in Table 17). The population comprised of 6 neutered males, 6 entire males, 8 neutered females and 4 entire females. The age of patients at the time of sample collection ranged from 1 year and 1 month to 12 years and 2 months. Crossbreed dogs were the most prevalent breed with 9 cases, followed by Labrador Retriever, Staffordshire Bull Terrier and Pugs with 2 cases each.

The median age of dogs with septic neutrophilic inflammation was 5 years 4 months, whereas the median age of the diagnostic cases in all non-septic inflammation categories was 6 years 0 months. Blood results were obtained in 9 of the 20 diagnostic cases (as shown in Table 18), two of which were from results obtained from samples submitted to the referral laboratory. The remaining cases were from supplied results obtained via in-house veterinary practice point-of-care analysers. Two of the five cases with septic neutrophilic inflammation had a concurrent moderate neutrophilia (28.6 & $19.1 \times 10^9/L$ [RI, $3.0 - 12.0$]). One case also had evidence of a left shift and a mild monocytosis ($1.7 \times 10^9/L$ [RI, $0.0 - 1.3$]). One of the septic cases had no abnormalities found on haematology or biochemistry and the remaining two cases had no blood results supplied or requested. The non-septic

cases (n = 6) primarily had no haematologic or biochemical abnormalities. However, one case with eosinophilic inflammation also had a moderate eosinophilia ($4.1 \times 10^9/\text{L}$ [RI, 0.1 – 1.2]), along with increased hepatic enzyme activities (GLDH 28 IU/L [RI, 2-6]; ALT 507 IU/L [RI, 13 – 78]; ALP 131 IU/L [RI, 12 – 83]). One case with mixed inflammation had a mild lymphopenia ($0.7 \times 10^9/\text{L}$ [RI, 1.0 – 5.0]) and a mildly decreased total T4 concentration (11.6 nmol/L [RI, 15 – 55]), and a further case diagnosed with mixed inflammation had a mild hyperproteinaemia (TP 73 g/L [RI, 50 – 72]).

Table 17. Cases recruited for the prospective CRP study. Details of the breed, sex, and age of the patient are reported. The CRP results from the Gentian cCRP assay and the Fujifilm cCRP assay are detailed - the results are shown in mg/L units. Cases are sorted by cytologic diagnosis with the organisms isolated via microbiological culture listed for each case. *FN – Female neutered, FE – Female entire, MN – Male neutered, ME – Male entire. WHWT – West Highland White Terrier, SBT – Staffordshire Bull Terrier, JRT – Jack Russell Terrier, CKCS – Cavalier King Charles Spaniel.*

Breed	Sex	Age	Gentian CRP	Fujifilm CRP	Cytologic Diagnosis	Organisms Isolated
Golden Retriever	FN	1 year 7 months	106.01	104.00	Septic neutrophilic inflammation	Beta-haemolytic streptococcus, E. coli
Italian Spinone	FE	11 years 6 months	101.14	102.00	Septic neutrophilic inflammation	No organisms
Giant Schnauzer	FE	1 year 7 months	106.15	100.00	Septic neutrophilic inflammation	Streptococcus spp.
Crossbreed	ME	1 year 2 months	30.27	43.00	Septic neutrophilic inflammation	Non-lactose fermenting coliform
Crossbreed	MN	10 years 9 months	29.34	30.00	Septic neutrophilic inflammation	Enterococcus spp.
Crossbreed	FN	11 years 0 months	18.40	20.00	Non-diagnostic	No organisms
Labrador	MN	9 years 7 months	< 6.8	7.00	Non-diagnostic	No organisms
Border Collie	FN	12 years 2 months	< 6.8	3.00	Non-diagnostic	No organisms
Crossbreed	FN	1 year 1 month	< 6.8	3.00	Non-diagnostic	No culture
Pug	MN	11 years 0 months	< 6.8	< 3.00	No cytologic abnormalities found	No culture
CKCS	ME	1 year 4 months	15.67	12.00	Neutrophilic inflammation	No organisms
SBT	MN	10 years 6 months	12.32	32.00	Neutrophilic inflammation	No organisms
Pug	MN	7 years 1 month	8.70	7.00	Neutrophilic inflammation	No organisms
Labrador	MN	2 year 9 months	< 6.8	6.00	Neutrophilic inflammation	No organisms
Crossbreed	FN	6 years 8 months	< 6.8	4.00	Neutrophilic inflammation	No culture
Yorkshire Terrier	ME	9 years 10 months	< 6.8	7.00	Macrophagic inflammation	Bordetella bronchiseptica
WHWT	FN	10 year 1 month	< 6.8	3.00	Macrophagic inflammation	No organisms
JRT	FE	1 year 10 months	18.41	25.00	Mixed inflammation	Angiostrongylus vasorum
Springer Spaniel	FN	9 years 10 months	24.49	23.00	Mixed inflammation	Non-lactose fermenting coliform
SBT	FE	12 years 0 months	15.97	20.00	Mixed inflammation	E. coli, Pseudomonas spp., Beta-haemolytic streptococcus
Crossbreed	ME	1 year 9 months	< 6.8	6.00	Mixed inflammation	No organisms
Crossbreed	FN	2 years 6 months	< 6.8	4.00	Mixed inflammation	No culture
Crossbreed	ME	8 years 0 months	11.39	16.00	Eosinophilic inflammation	No organisms
Crossbreed	ME	1 year 1 month	< 6.8	3.00	Eosinophilic inflammation	No organisms

Table 18. Summary table showing the nine CRP cases from *Table 18* with blood results. CRP results are shown in mg/L units. *NAD – no abnormalities detected.*

Case	Gentian CRP	Fujifilm CRP	Cytologic Diagnosis	Organisms Isolated	Blood results
1	106.01	104.00	Septic neutrophilic inflammation	Beta-haemolytic streptococcus, E. coli	Neutrophils $28.6 \times 10^9/L$ (3.0 - 12.0) Band neutrophils $1.2 \times 10^9/L$ (0-0.3) Monocytes $1.7 \times 10^9/L$ (0 - 1.3)
2	101.14	102.00	Septic neutrophilic inflammation	No organisms	Neutrophils $19.1 \times 10^9/L$ (3.0 - 12.0)
5	29.34	30.00	Septic neutrophilic inflammation	Enterococcus spp.	NAD
12	12.32	32.00	Neutrophilic inflammation	No organisms	NAD
15	< 6.8	4.00	Neutrophilic inflammation	No culture	NAD
19	24.49	23.00	Mixed inflammation	Non-lactose fermenting coliform	Total protein 73 g/L (50-72)
20	15.97	20.00	Mixed inflammation	E. coli, Pseudomonas spp., Beta-haemolytic streptococcus	Lymphocytes $0.7 \times 10^9/L$ (1.0 - 5.0) Total T4 11.6 nmol/L (15-55)
21	< 6.8	6.00	Mixed inflammation	No organisms	NAD
24	< 6.8	3.00	Eosinophilic inflammation	No organisms	Eosinophils $4.1 \times 10^9/L$ (0.1-1.2) GLDH 28 IU/L (2-6) ALT 507 IU/L (13-78) ALP 131 IU/L (12-83)

4.2.2 Cytological findings

Cytologic examination was performed by two board-certified clinical pathologists at Finn Pathologists. Cases were categorised into one of the cytologic diagnoses detailed in Table 8. Five cases of septic neutrophilic inflammation were diagnosed, with five cases of neutrophilic inflammation (without sepsis), and five cases of mixed inflammation. Two cases of eosinophilic inflammation were diagnosed, with two cases of macrophagic (mononuclear) inflammation. One case showed no cytologic abnormalities. Four cases were considered non-diagnostic, and therefore not included within the study for further analysis. One case diagnosed with mixed inflammation was also found to have a lungworm infestation, with larvae visualised on bronchoalveolar lavage fluid cytology. The mixed inflammation was a

combination of eosinophilic and neutrophilic inflammation. One case of neutrophilic inflammation was presumed to have a *Pneumocystis sp.* infection based on visualisation of suspected *P. carinii* cysts on BAL cytology. However, two PCR tests for *Pneumocystis sp.* on BAL fluid (only one submitted for cytology) showed negative results, and therefore Pneumocystosis was not confirmed and presumed to be absent.

4.2.3 Microbiological findings

Microbial culture was requested in 19 of 24 cases (79%), with a positive culture result in 7 of those cases. 5 of the 7 cases with a positive culture had one organism isolated, with one case yielded two organisms and a second with three organisms isolated. *E. coli*, non-lactose fermenting coliforms and Beta-haemolytic *Streptococcus sp.* were the most common organisms isolated. One case with mixed inflammation was diagnosed with *Angiostrongylus vasorum* parasitism after visualisation of lungworm larvae on lavage cytology, confirmed with a positive serology result.

One case of septic neutrophilic inflammation yielded no organisms on microbial culture, however intracellular bacteria were noted on cytology and therefore the case was categorised as septic. Conversely, two cases of mixed inflammation yielded a positive culture, one with non-lactose fermenting coliforms, and a second with multiple isolates cultured – *E. coli*, *Pseudomonas spp.* and Beta-haemolytic *Streptococcus*. *Bordetella bronchiseptica* was cultured with one case with a diagnosis of mononuclear inflammation.

4.2.4 CRP analysis

C-reactive protein concentrations ranged from <6.8 mg/L, designated as 3.4 mg/L as described earlier, to 106.15 mg/L as measured by the Gentian cCRP assay. Concentrations of CRP measured by the Fujifilm cCRP assay ranged from < 3 mg/L to 104 mg/L. Cases diagnosed with septic neutrophilic inflammation had CRP concentrations from 29.34 mg/L to 106.15 mg/L, with a mean value of 75 or 76 mg/L when measured with the Gentian or Fujifilm cCRP assays respectively. Non-septic cases had CRP concentrations up to 32 mg/L measured with the Fujifilm cCRP assay or 24.49 mg/L with the Gentian cCRP assay. The mean values of CRP were significantly different between cytologic diagnoses ($p = < 0.001$) when analysed with a one-way ANOVA, and there were statistically significant differences between sepsis cases and each of the other diagnoses when measured with a Tukey's multiple comparisons test ($p = 0.039$ to 0.002 ; Figure 7). There was no statistical difference in CRP results between the two assays for each category of cytologic diagnosis when analysed with a Wilcoxon matched pairs signed rank test.

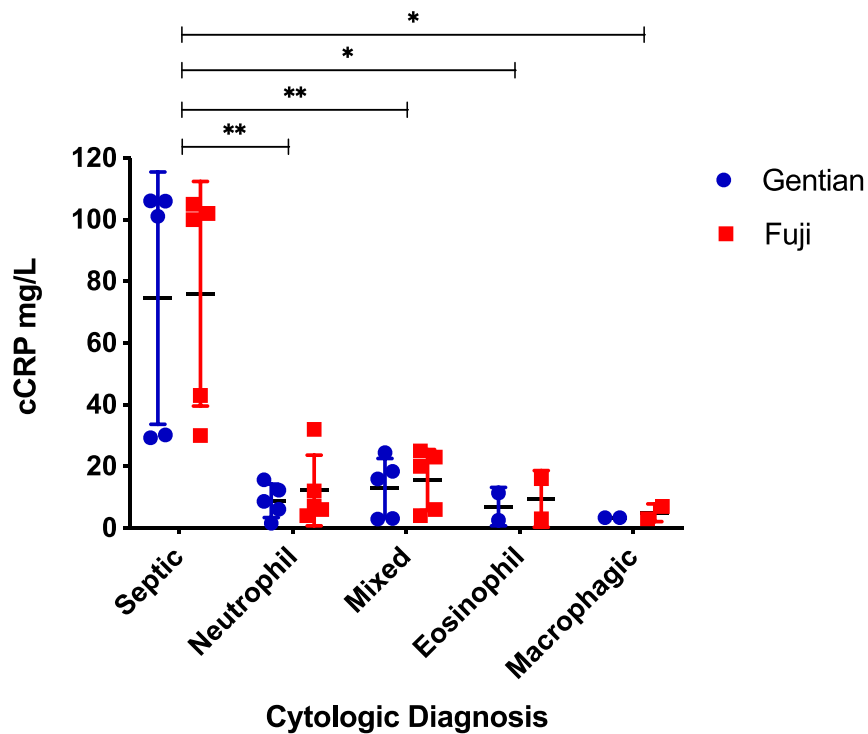


Figure 7 – CRP concentrations in mg/L measured by the Gentian cCRP assay (Gentian) and Fujifilm cCRP assay (Fuji) for cases diagnosed with septic neutrophilic inflammation (Septic), neutrophilic inflammation (Neutrophil), mixed inflammation (Mixed), eosinophilic inflammation (Eosinophil), or macrophagic inflammation (Macrophagic) by BAL fluid cytology. * = $p < 0.05$, ** = $p < 0.01$.

4.2.5 Assessment of diagnostic utility

Receiver-operator characteristic (ROC) curves were constructed to analyse the diagnostic accuracy of CRP using the two different tests (Figure 8). Sensitivity, specificity, likelihood ratios and areas under the curve were calculated. Based on the findings from this study, the sensitivity and specificity of the Gentian cCRP assay to diagnose or exclude sepsis were both 100% at 27 mg/L (95% CI = sensitivity 57.0 – 100%; specificity 78.5% - 100%). The area under the curve (AUC) was 1.00 ($p = 0.001$). In comparison, the Fujifilm cCRP assay also appeared most optimal at 27 mg/L, with a sensitivity of 100% (95% CI 57.0 – 100%) and a specificity of

92.86% (95% CI 68.53 – 99.63%). The positive likelihood ratio at this concentration was 14.0.

The negative likelihood ratio could not be calculated with a 100% sensitivity. AUC for the Fujifilm assay was 0.986 (95% CI 0.942 – 1.000) ($p = 0.002$).

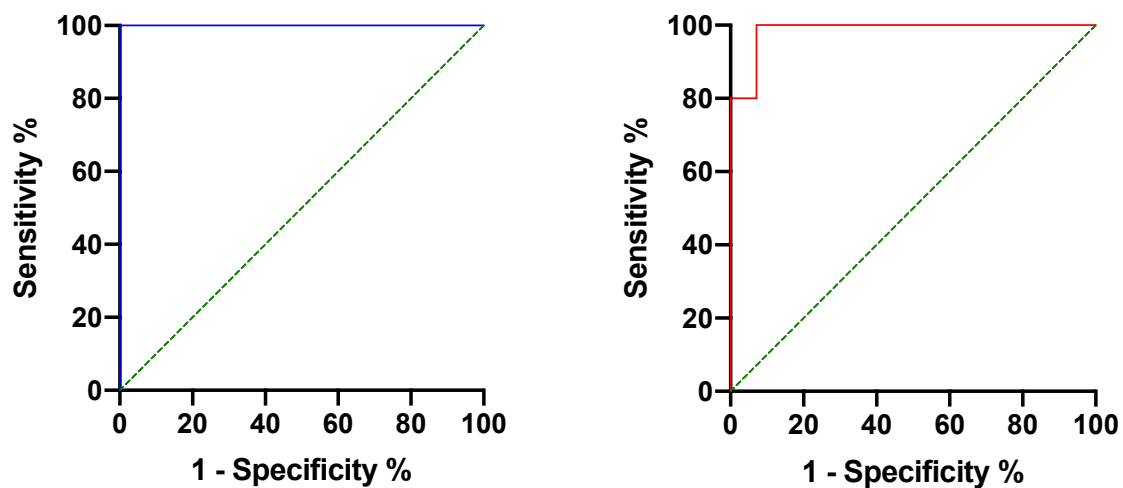


Figure 8. A – ROC curve for canine CRP in canine serum measured with the Gentian cCRP assay. AUC – 1.00. **B** – ROC curve for canine CRP in canine serum measured with the Fujifilm cCRP assay. AUC – 0.986. For both plots the dotted green line represents the identity line.

A contingency table was constructed (Table 19) to summarise the number of septic vs non-septic cases with and without a CRP concentration above the clinical decision limit of 27 mg/L. From this, the positive and negative predictive values could be easily calculated at the study prevalence of 20%.

Table 19. Compound contingency table detailing the number of true and false positive and negative cases for both assays utilising the proposed clinical decision limit of 27 mg/L.

	Septic inflammation cases	Non-septic cases	
CRP concentration > 27 mg/L	Gentian - 5 Fujifilm - 5	Gentian - 0 Fujifilm - 1	PPV = Gentian – 100% Fujifilm – 83.33%
CRP concentration ≤ 27 mg/L	Gentian - 0 Fujifilm - 0	Gentian - 15 Fujifilm - 14	NPV = Gentian – 100% Fujifilm – 100%

4.3 Part 3 – Method Comparison Study

A total of 123 patient serum samples were collected for the comparison study. Patients were randomly selected regardless of breed, age, sex or clinical history. The results of the precision study showed that imprecision of Fujifilm cCRP was lower than the allowable imprecision of 12.2% defined in a previous study of CRP biologic variation (Kjelgaard-Hansen *et al.*, 2003). The low serum concentration pool (13.5 mg/L) showed within-run imprecision (CV%) of 10.2%, whereas the high serum concentration pool (63.3 mg/L) showed imprecision of 1.63%. Between run CV% for low and high serum pools was 4.3% and 1.2% respectively. The study of linearity under dilution (Figure 10) showed small proportional and constant errors. The slope was 1.024 (95% CI 0.925-1.123) and the y-intercept was -1.50 mg/L (95% CI -4.77 – 1.77 mg/L). Linearity showed good correlation ($r^2 = 0.986$) between expected and measured values.

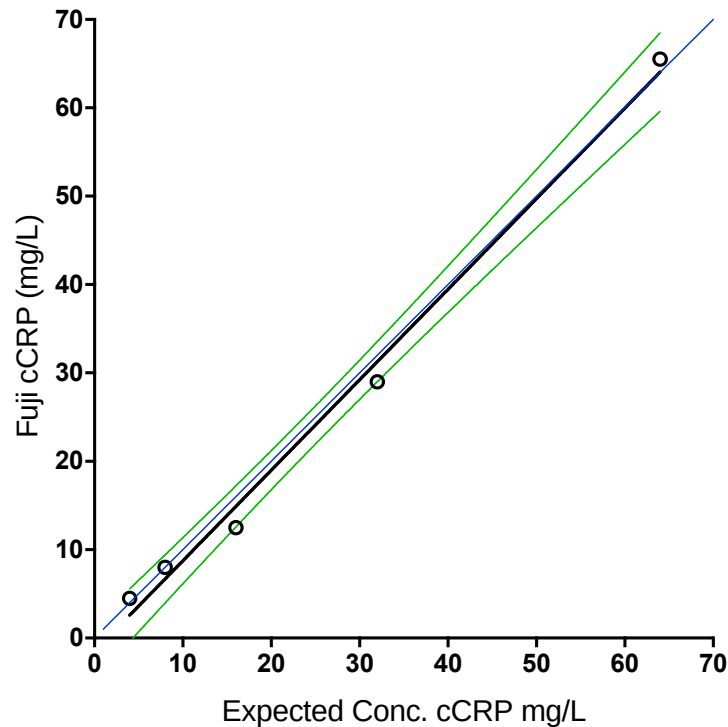


Figure 10 – Linearity under dilution of a canine serum sample with a cCRP concentration of 64 mg/L. Dilutions of 0.5, 0.25, 0.125 and 0.0625 of the original concentration were measured with the Fujifilm cCRP assay. The solid black line represents the regression line ($y = 1.024x + 1.5 \text{ mg/L}$) ($r^2 = 0.986$). The green lines represent the 95% confidence intervals. The blue line represents $y = x$.

The method comparison study showed non-normally distributed data when analysed with Shapiro-Wilk normality testing. As a result, a Spearman correlation was performed, which showed good correlation ($r = 0.886$). The correlation was < 0.975 , therefore a Deming regression was performed with SD variables from both assays included as the defined variation. The regression analysis showed a predominantly proportional error (Figure 11), with a slope of 1.323 (95% CI of 1.27 – 1.37), with a small constant error reflected by an intercept of 1.0 mg/L (95% CI 0.01 – 2.0). The Bland-Altman plots (Figure 12) also predominantly show a positive proportional bias, with a mean bias of 10.54 mg/L (95% agreement limits -23.15 – 44.22 mg/L), or a mean percentage bias of 39.9%. At a proposed

clinical decision CRP concentration of 27 mg/L (from the results of the CRP study detailed in the previous section), the Fujifilm cCRP assay would yield a result of 36.7 mg/L using the regression equation [$y = 1.323x + 1.0$ mg/L], a bias of 9.7 mg/L or a percentage bias of 36%.

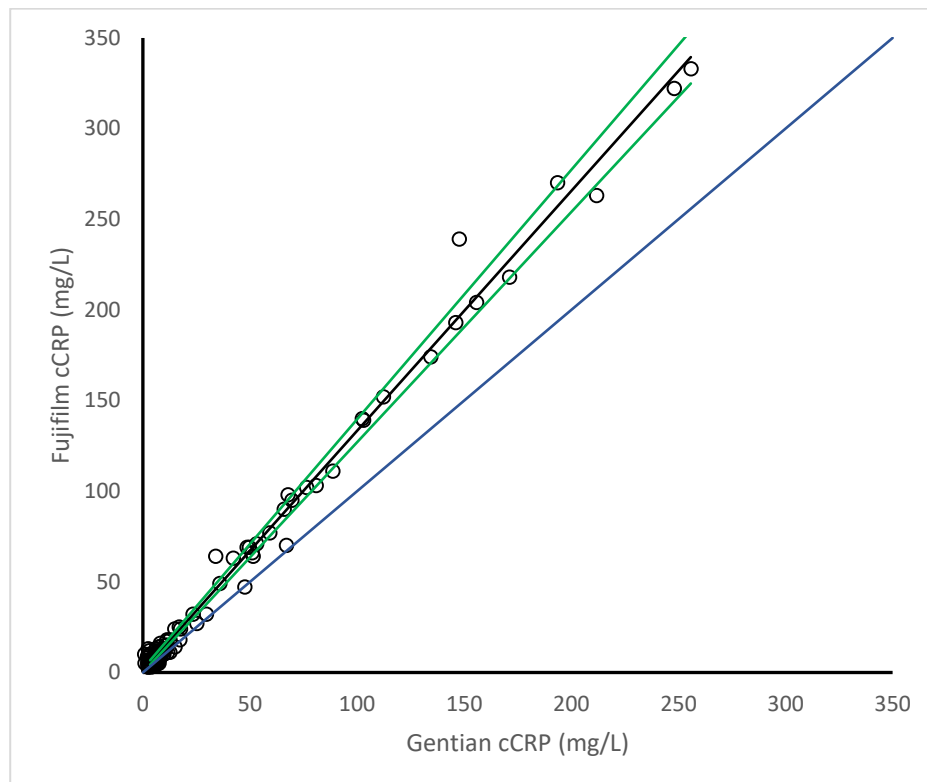


Figure 11 – Deming regression analysis for CRP in canine serum samples measured with the Fujifilm cCRP assay in comparison with the Gentian cCRP assay. The solid black line represents the regression line ($y = 1.323x + 1.0$ mg/L). The green lines represent the 95% confidence intervals. The blue line represents $y = x$.

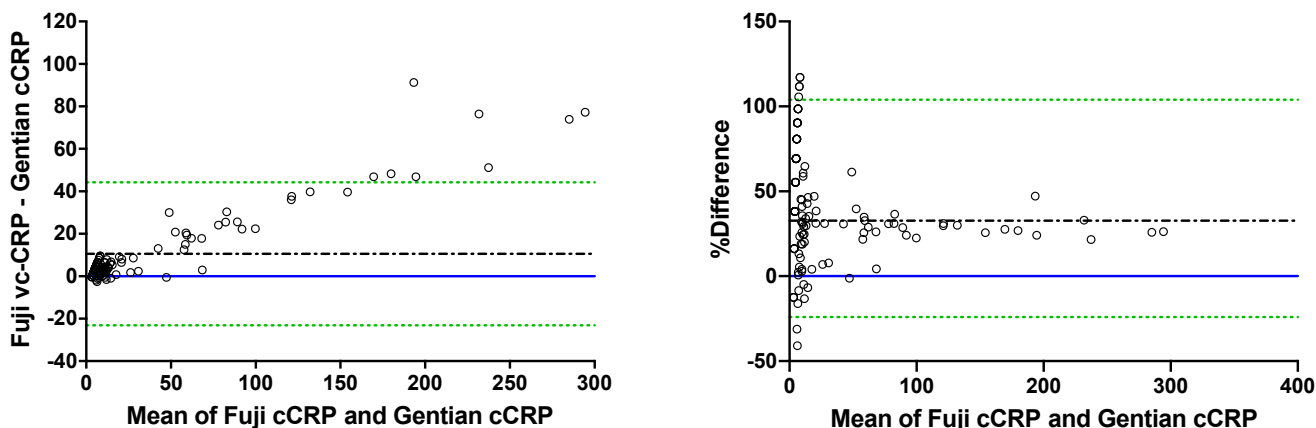


Figure 12. A – Bland-Altman difference plot for CRP in canine serum measured with Fuji cCRP in comparison with the Gentian cCRP assay. A mean bias of 10.54 mg/L is present. **B** – Bland-Altman percentage difference plot for CRP in canine serum measured with Fuji cCRP in comparison with the Gentian cCRP assay. A mean %bias of 39.9% is present. For both plots the dotted black line represents the mean bias, absolute bias in **A** and % bias in **B**. The green lines represent the limits of agreement, defined as the mean difference $\pm$ 1.96 times the SD. The blue line represents the identity line.

Total error was assessed at a clinically relevant CRP concentration of 27 mg/L. Combining both the % bias and imprecision (CV%) to calculate TE_{obs} the result showed an error of 41.5%, which is higher than the desirable TE_a of 29.6%, although the result is less than the minimum TE_a of 44.4%. Method decision (MEDx) charts (Figures 13 & 14) and Sigma metrics were calculated for the assay. The Sigma metric for a TE_a of 29.6% was below the required stable operating minimum of 2 sigma. When compared against the minimum TE_a of 44.4%, the assay appears to perform at 3 sigma, which would be considered acceptable analytical performance.

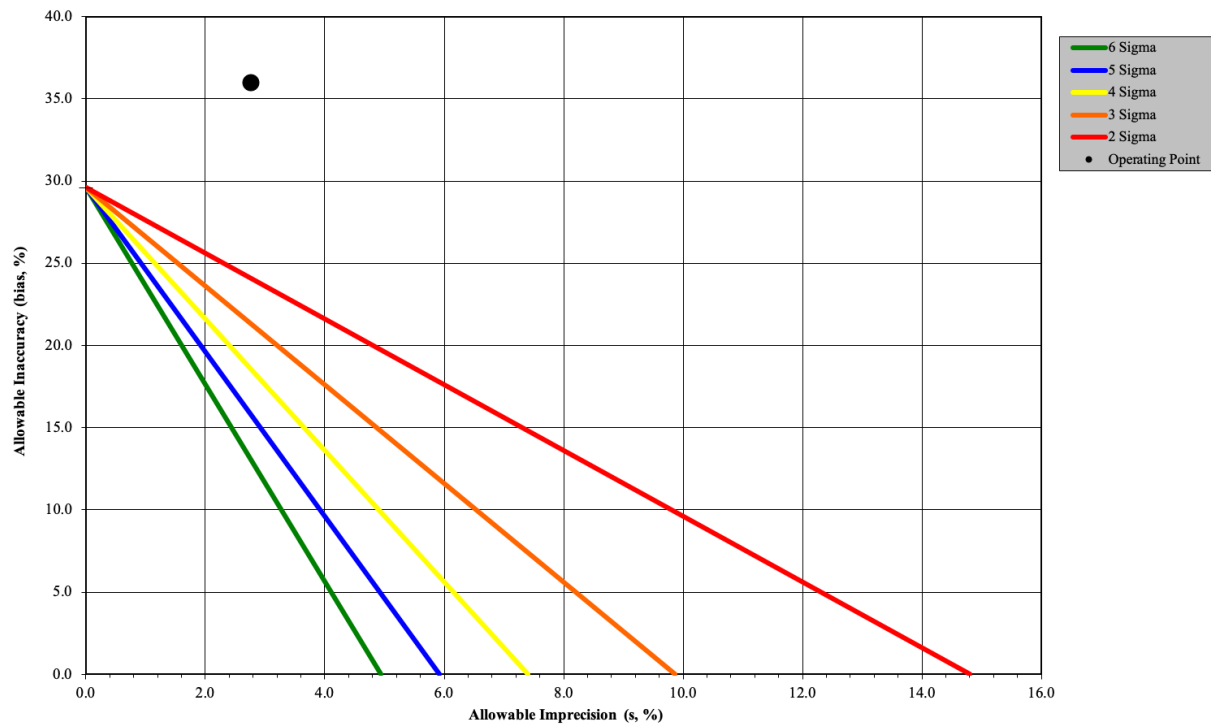


Figure 13 – MEDx method decision chart for the Fuji cCRP assay. The desirable allowable total error is defined as 29.6%. Inaccuracy is plotted against imprecision of the assay. Operating limits for different Sigma performance levels are plotted as coloured lines. The operating point (black dot) is the current performance of the assay within the allowable error requirements. The operating point is beyond the stable operating limit of the assay (above the red 2 Sigma line).

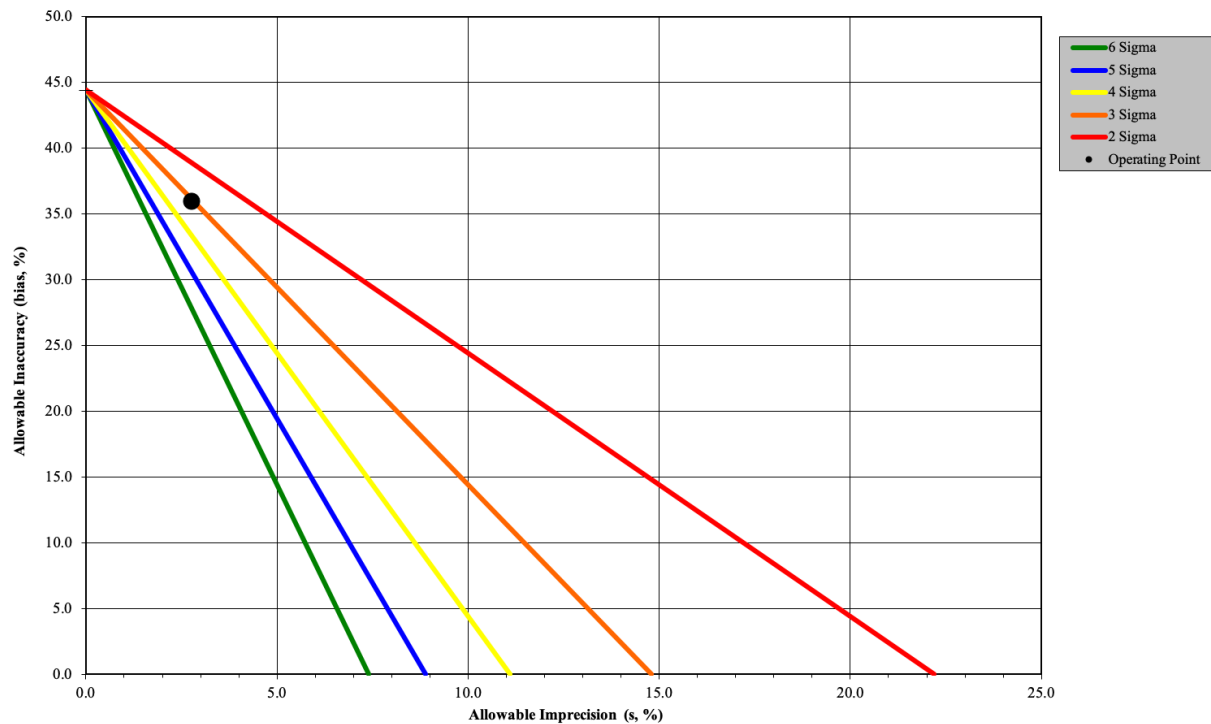


Figure 14 – MEDx method decision chart for the Fuji cCRP assay. The minimum allowable total error is defined as 44.4%. The operating point of the assay has shifted with the less stringent total error requirements and is operating at 3 Sigma (below the orange 3 Sigma line).

The results show the total error appears largely due to a proportional bias between the methods, with a small constant bias and a good level of precision. Therefore, to explore the effect of reduced proportional bias on the results and allow for results to be comparable between methods in a clinical setting, a correction factor of +30% was applied to the Gentian cCRP results and the Deming regression repeated, which showed improved results, with an almost eliminated proportional error and similar small constant bias (Figure 15). The regression yielded a slope of 0.992 (95% CI of 0.952 – 1.031), with an intercept of 1.06 mg/L (95% CI 0.046 – 2.075). The Bland-Altman plots (Figure 16) also show a reduced positive proportional bias, with a mean bias of 0.73 mg/L (95% agreement limits -11.64 – 13.10

mg/L), or a mean percentage bias of 12.5%. Using the regression equation [$y = 0.992x + 1.06$ mg/L] at a clinical decision level of 27 mg/L, there is a bias of 0.9 mg/L or a percentage bias of 3.4%.

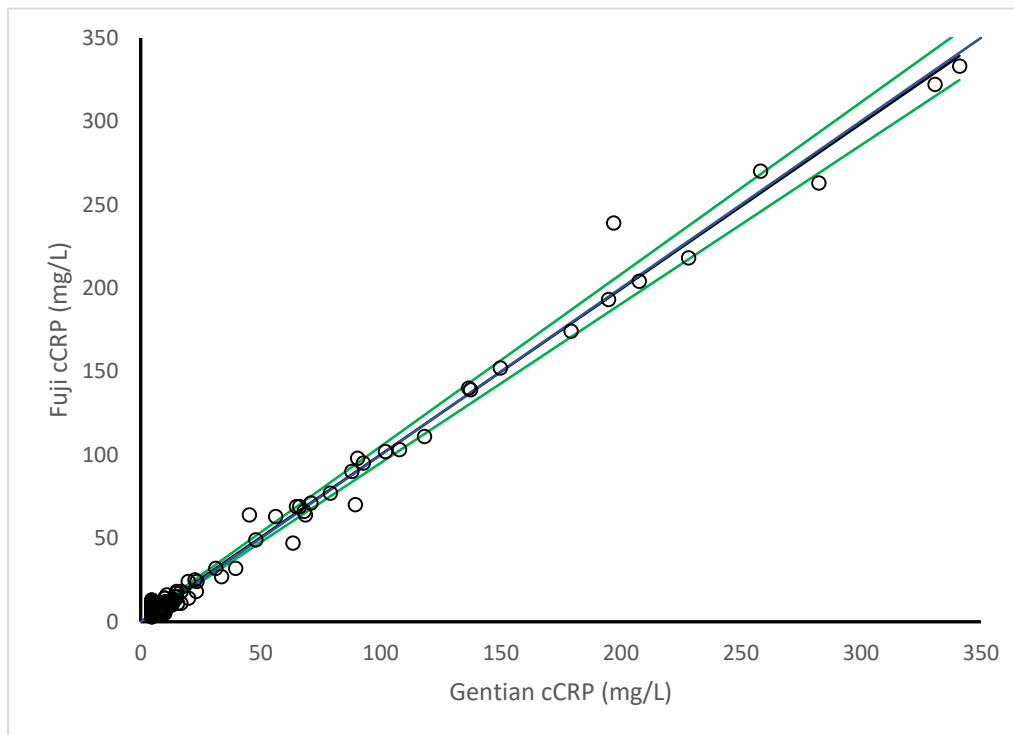


Figure 15 – Deming regression analysis for CRP in canine serum samples measured with the Fuji cCRP assay in comparison with the Gentian cCRP assay with an added correction factor of +30%. The solid black line represents the regression line ($y = 0.992x + 1.05$ mg/L). The green lines represent the 95% confidence intervals. The blue line represents $y = x$.

Total error (TE_{obs}) at 27 mg/L was 8.9%. The TE_{obs} is less than both the $desTE_a$ and $minTE_a$ with this correction factor. Method decision (MEDx) charts (Figures 17 & 18) were created with the added correction factor for the Gentian cCRP assay. The Sigma metrics for a TE_a of 29.6% and a TE_a of 44.4% were both greater than 6 Sigma at 9.5 and 14.9 respectively, which is considered world-class performance.

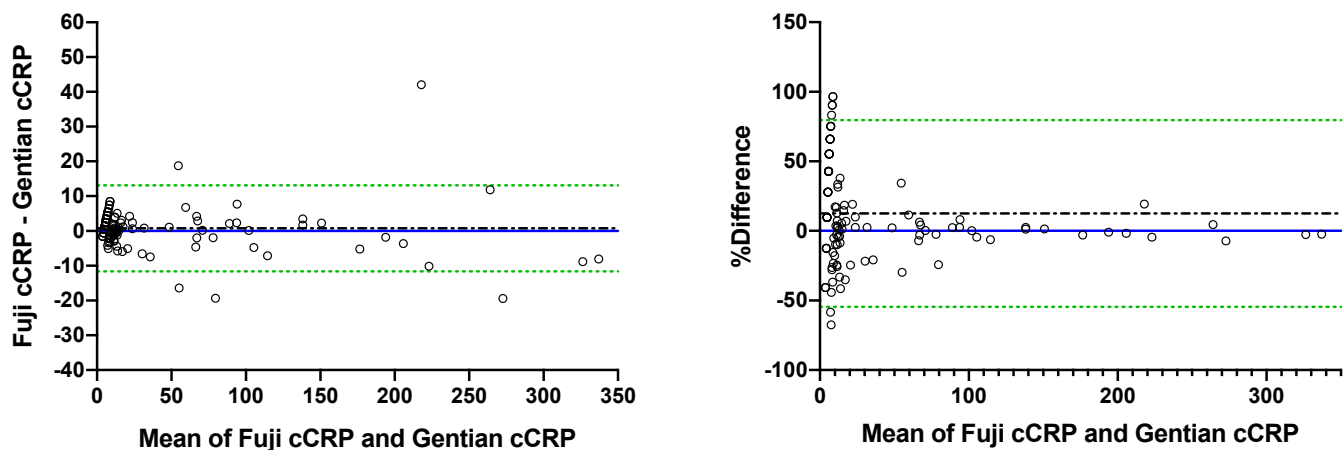


Figure 16. A – Bland-Altman difference plot for CRP in canine serum measured with Fuji cCRP in comparison with the Gentian cCRP assay with a correction factor of +30%. A mean bias of 0.73 mg/L is present. **B** – Bland-Altman percentage difference plot for CRP in canine serum measured with Fuji cCRP in comparison with the Gentian cCRP assay with a correction factor of +30%. A mean %bias of 12.5% is present.

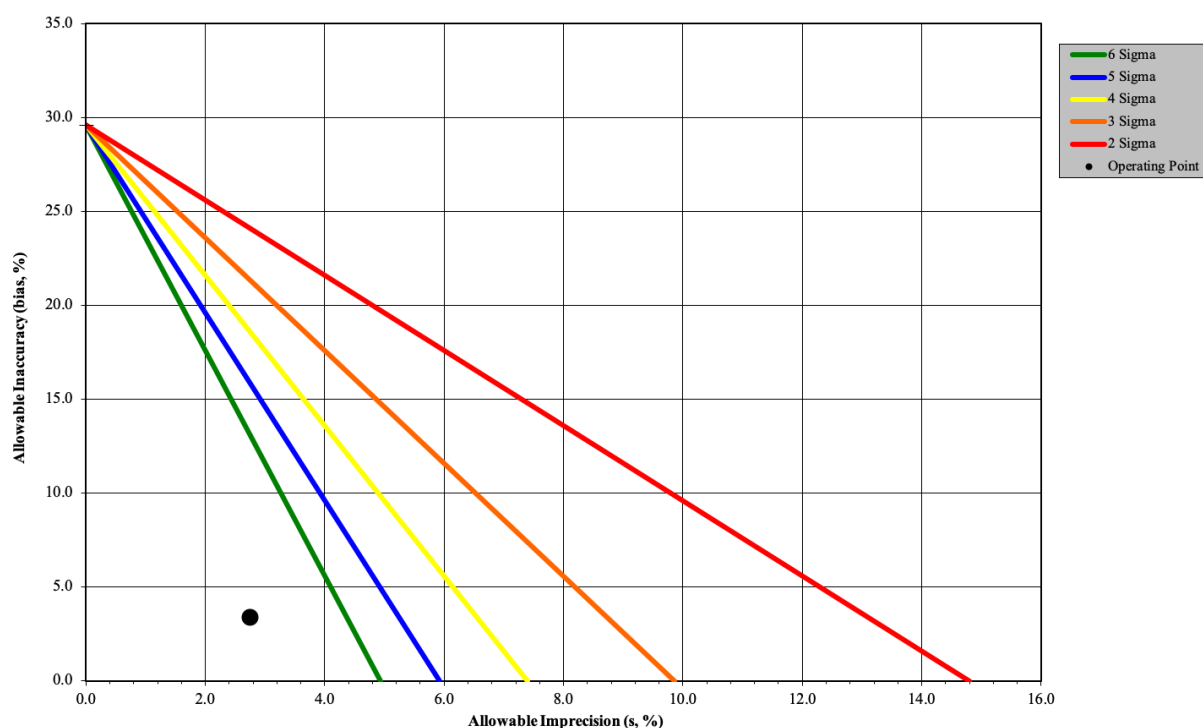


Figure 17 – MEDx method decision chart for the Fuji cCRP assay with the 30% correction factor on the comparator Gentian cCRP assay. The allowable total error is defined as 29.6% (the desired TE_a). The operating point is below the green 6 Sigma line, indicating the assay is operating at higher than 6 Sigma performance with the added correction factor.

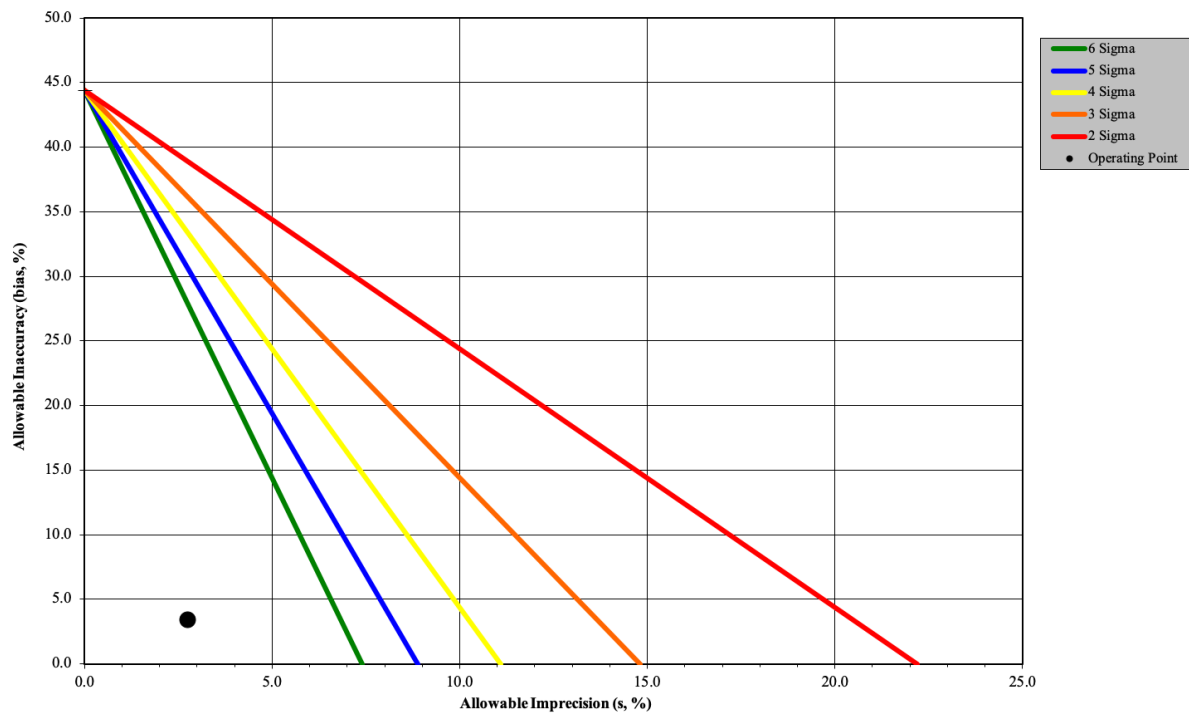


Figure 18 – MEDx method decision chart for the Fuji cCRP assay with the 30% correction factor on the comparator Gentian cCRP assay. The allowable total error is defined as 44.4% in this case (minimum TE_a). The operating point is below the green 6 Sigma line, indicating the assay is operating at or above 6 Sigma with the added correction factor, which would be considered optimum performance.

5. Discussion

5.1 Part 1 – Retrospective Epidemiological study

The main aims of collecting retrospective data on canine BAL submissions were to investigate the prevalence of infectious lower respiratory tract disease in the population, along with other potential cytologic diagnoses, and to determine the methodology used to diagnose lower respiratory tract infections. The retrospective data collected from canine BAL submissions to the three CVS laboratories over the allotted time period included a total of 2501 cases, of which 69.4% had concurrent microbial culture requested with the submission. The reason for microbial culture not being requested in as many as 30.6% of submissions is not clear, with no published data from other studies available to suggest what proportion of airways submissions to referral laboratories also have microbial culture requested. Factors for clients not requesting microbial culture may involve financial constraints, insufficient sample quantity, or perceived lack of benefit in cases already receiving antimicrobial therapy. Optimal diagnosis of bacterial pneumonia or lower respiratory tract infections requires confirmation of septic inflammation on airway cytology obtained through BAL and a positive microbial culture, along with resolution of clinical signs following appropriate antimicrobial therapy (Dear, 2014). The prevalence of cases diagnosed with septic neutrophilic inflammation (septic) on BAL cytology, and therefore presumed to have a lower respiratory tract infection was 16.2% of cases, although this prevalence increases to 17.9% if only diagnostic cases are included. Seventy-three percent of those septic cases with microbial culture results available had a concurrent positive microbial culture, confirming the diagnosis. However, the remaining 27% of cases had no

organisms isolated on culture. The cases in each of the other categories of cytologic diagnosis had predominately negative cultures, although still had a high proportion of cases with positive culture results.

Potential causes for the negative culture results in septic cases may be due to poor viability of the organisms or their death in transit to the laboratory. Other causes of bacterial death or non-viability may be due to the length of time between sample collection and sample culture. Many cases are received and processed within 24 hours, however many are not received at the laboratory within this time period, particularly at weekends and holidays. A further possible cause may be that the pathologic organism may be atypical and not identified on routine culture. *Mycoplasma spp.* infections are of particular concern with respiratory tract infections, and are an important and underdiagnosed primary respiratory pathogen that require complex specialist media for reliable culture (Priestnall *et al.*, 2014). *Mycoplasma* may be of further importance due to their suspected association with concurrent *B. bronchiseptica* infections, and the often increased severity of disease when these organisms are implicated (Randolph *et al.*, 1993; Chalker *et al.*, 2004).

Bacteria cultured in cases without cytologic signs of sepsis and with other cytologic interpretations may be present as a result of sample contamination, oropharyngeal contamination, or a reduction of bacterial numbers visible on a cytology smear due to prior antimicrobial therapy. Sequestration of bacteria within the respiratory tract is another possibility, which has been visualised with *Bordetella bronchiseptica* infections (Johnson *et al.*, 2013), leading to fewer intracellular bacteria exfoliated in airway wash samples. The diagnostic quality of fluid cytology samples may also impact the cytological recognition of

bacteria. Such factors may include large amounts of mucus or cellular debris obscuring cellular visualisation and decreasing the possible identification of intracellular bacteria, poorly preserved or tightly packed cells may also be further causes. A further consideration is the potential for overgrowth of bacteria due to increased storage times and temperature. One study demonstrated that inappropriate storage at room temperature for more than 24 hours led to an overgrowth of both *E.coli* and *B. bronchiseptica* in canine BAL fluid samples to levels consistent with infection (Curran *et al.*, 2020).

The bronchoalveolar lavage procedure is not considered to be sterile, therefore the reliability of microbial culture results may be questionable in many cases. Oropharyngeal contamination can be visualised on cytology with the presence of squamous epithelial cells, often with associated bacteria, or can be confirmed from the presence of oropharyngeal commensals such as *Simonsiella spp.* bacteria. In this study, if oropharyngeal contamination was included in the cytologic interpretation then the case was excluded, however some cases may have had evidence of oropharyngeal contamination described but was not included in the interpretation.

The most frequent bacterial isolates from each group were a mixed infection, which corroborated the findings from a previous study of bacterial culture and cytology from BAL specimens (Peeters *et al.*, 2000). The most frequently cultured single organisms isolated in cases with a cytologic diagnosis of septic inflammation were *Bordetella bronchiseptica*, *Pasteurella sp.*, *E. coli* and other coliforms; and the most frequently isolated bacteria in cases without a cytologic diagnosis of sepsis were *Pasteurella*, *E. coli* and *B. bronchiseptica*. In the same study by Peeters *et al.* the most frequently isolated organisms in lower

respiratory infection cases in order of frequency were *Pseudomonas*, *Streptococcus* sp. and *B. bronchiseptica*. These differences may be explained however by the limited sample size of 13 cases in that study, which limits direct comparison.

Assessment of signalment data from the large dataset yielded showed that the average age of septic cases (7 years 11 months) was significantly lower than the average age of cases with no abnormalities (8 years 11 months), and lower than the average ages of other categories also, but without statistical significance. The average age of septic cases in this study was higher than those reported in a previous study of dogs with lower respiratory tract infection, with a mean of 3 years (Johnson *et al.*, 2013). A previous study of bacterial culture and BAL cytology of dogs with respiratory tract disease found that dogs with chronic bronchitis were on average significantly older than those with infectious respiratory disease, although no figures were quoted (Peeters *et al.*, 2000). Cases diagnosed with eosinophilic inflammation were on average much younger than those diagnosed with other abnormalities or compared with normal cases, with a median age of 4 years 11 months. This may reflect a higher prevalence of allergic or parasitic diseases in younger animals. These findings are similar to those in a previous study of CRP and respiratory disease (Viitanen *et al.*, 2014) which showed an average age of 4.2 years for cases of eosinophilic bronchopneumopathy, although this was higher than the average age of bacterial pneumonia cases in this study (3.9 years). There was no apparent sex predilection, and neutering status appeared to have no impact.

The most prevalent breed from the canine BAL submissions was crossbreed dogs, with significant differences in the prevalence of many breeds compared with the population of

dogs in the total laboratory submissions, used as a standard reference population. The prevalence of BAL submissions was compared with the reference laboratory caseload from the three laboratories as a whole. A comparison with each individual laboratory population was not formally assessed, and although each laboratory has a similar practice demographic (i.e. proportion of first opinion and referral practices) from a broad geographic region, population-related differences may be present in the data. The prevalence of dogs in cytologic disease categories are similar in many cases to the wider population of BAL submissions, however certain anomalies are noted. In cases diagnosed with septic neutrophilic inflammation, Bulldogs accounted for 4% of the population, compared with 1.4% of the total BAL caseload, or 0.58% of total laboratory submissions. No predisposition to infectious respiratory disease was found in the literature for this breed, however a possible link to brachycephalic obstructive airway syndrome or brachycephalic conformation may exist. Siberian Huskies were the third most common breed diagnosed with eosinophilic inflammation, with 10% of cases. This compared with 1.72% of all BAL cases or 0.56% of total submissions. Siberian Huskies are known to be predisposed to pulmonary eosinophilia (Clercx *et al.*, 2000; Lilliehöök and Tvedten, 2003), which may account for the increased prevalence in this category.

Odds ratios were calculated to further assess the influence of breed on the likelihood of different cytologic diagnoses (Table 15). English springer spaniels and Yorkshire Terriers appear to be associated with higher odds of infectious disease compared with the control population of crossbreed dogs. Yorkshire Terriers also had increased odds of a diagnosis of macrophagic inflammation, although these findings should be interpreted with some caution as a relatively large 95% confidence interval was noted, likely due to the small

number of cases with this diagnosis. No association between these breeds and infectious respiratory disease were found in the published literature, and so further investigation is warranted. Siberian Huskies and Whippets showed increased odds of eosinophilic inflammation; however, once again the wide 95% confidence intervals likely reflect the low number of individual cases reducing the confidence in the result. An association between eosinophilic bronchopneumopathy and Siberian Huskies has been reported, as described above, however no known link has been shown with Whippets. Conversely, Border Collies appeared to show lower odds of both neutrophilic and mixed inflammation.

Further investigation of the impact of brachycephalic conformation in associated breeds showed no significant differences in the prevalence of each cytologic diagnosis when compared with the study population as a whole. However, a greater percentage of cases with septic inflammation was noted (22.05%) compared with the caseload as a whole (16.2%). Although upper respiratory disease is frequently reported in these dogs, only an increased incidence of aspiration pneumonia in brachycephalic breeds has been published in peer-reviewed studies, with no published data on the prevalence of other infectious or non-infectious lower respiratory disease in these breeds (Hoareau, Mellema and Silverstein, 2011; Packer *et al.*, 2019). Bacterial isolates cultured from BAL fluid in these cases were of a similar frequency compared to the study population as a whole, with a mixed infection the most common result. *B. bronchiseptica* was the most frequently isolated single organism, which may reflect the increased percentage of sepsis cases in these breeds.

The effect of a rural or urban location on potential cytologic diagnosis was analysed using the postcode of the submitting practice and comparison with the Office for National

Statistics (ONS) postcode census data. The majority of cases were from practices in an urban location, which is likely to reflect the typical population distribution across the country, rather than being a significant increase in overall cases from an urban environment. No significant differences were found between those from an urban or rural environment for any cytologic diagnosis. Dogs from urban environments may be more likely to be exposed to pollutants, and there is evidence of increased susceptibility to allergic disease in dogs and people living in urban environments (Hakanen *et al.*, 2018). A limitation of the methodology for this data is that the postcode of the veterinary practice from which cases were submitted were used to classify cases, as no details of the home addresses of owners were collected by the laboratories. This limited the analysis and means the results are only an approximation, because the exact home location of each case could not be assessed, and the environment of the veterinary practice may differ from the home environment of the patient. A further limitation is that cases submitted from referral practices were not likely to represent the home environment of the patient as clients may travel long distances to seek specialist care, however referral cases were not excluded from the analysis.

5.2 Part 2 – Prospective CRP study

The aim of this study was to assess if CRP could be used as a decision-making tool to help determine appropriate prescription of antimicrobials in cases of lower respiratory tract infections in dogs. The results showed a statistically significant difference in CRP concentration between septic cases and those without a cytologic diagnosis of sepsis. CRP

concentrations in septic cases ranged from 29.34 mg/L to 106.2 mg/L, with the majority of non-septic cases yielding CRP concentrations below 25 mg/L, excluding a maximum value of 32 mg/L for one case with neutrophilic inflammation. The mean concentration of CRP in septic cases (75 mg/L Gentian, 76 mg/L Fujifilm) was statistically different from all other disease categories, which appeared to support previous findings by Nakamura *et al.*, 2008 and Viitanen *et al.*, 2014, who showed a significant increase in CRP concentration for cases with bacterial pneumonia.

Interestingly, the CRP concentrations from the five cases diagnosed with septic neutrophilic inflammation appeared to be split into two groups. Three cases showed a CRP concentration of > 100 mg/L with both assays, and the remaining two cases yielded a concentration of 29.34 – 43 mg/L between the two methods (Figure 7). This separation may represent a different stage of infection with those cases with lower concentrations of CRP at either a very early stage, of later stage of infection with partially resolving infection and inflammation. The latter is considered more likely given the very short time between infection and induction of the acute phase response (24 hours), by which time the clinical signs may have only recently become apparent and a BAL procedure is unlikely to have yet been performed.

Intriguingly, the case diagnosed with *Angiostrongylus vasorum* infestation showed a mixed inflammation on cytology (with an increase in both neutrophils and eosinophils) and had a CRP concentration of 18.41 / 25 mg/L, suggesting a more mild stimulation of CRP production in this disease. However, it should be noted that this finding was from a single case. As a result this should be interpreted with caution as this change may be due to chance and

further data would be needed to investigate further. There is no known published data for CRP responses in *A. vasorum* or other lungworm infestations.

Both the Gentian and Fujifilm assays performed well in this study and the results of both assays appeared to show no statistical differences between mean values in each disease category. They were able to detect cases with sepsis with a 100% sensitivity at 27 mg/L, and the Gentian assay was able to discriminate those cases from non-septic inflammation with 100% specificity at this concentration. The Fujifilm assay showed a 92.86% specificity at the same concentration with a positive likelihood ratio of 14.0, suggesting that a CRP concentration above 27 g/L is 14 times more likely for a patient with septic neutrophilic inflammation compared to a patient without sepsis. A negative likelihood ratio was not possible to calculate for either method due to a 100% sensitivity. Both assays had a 100% negative predictive value (NPV), indicating that each test would exclude the possibility of septic inflammation below a CRP concentration of 27 mg/L. The positive predictive value (PPV) for the Gentian assay and Fujifilm assay suggests that a diagnosis of infection is predicted in 100% and 83.33% of cases respectively, in results exceeding the same CRP concentration. Given that a concentration of > 27 mg/L was observed in one non-infectious case, and both assays demonstrated negative predictive values and sensitivity values of 100% at this clinical decision limit, the use of CRP may be best used for excluding lower respiratory infections, as initially predicted, rather than for actively diagnosing infectious disease. Predictive values are also influenced by prevalence. As the prevalence increases, the PPV increases but the NPV decreases. Similarly, as the prevalence decreases the PPV decreases while the NPV increases. The prevalence of infectious respiratory disease in this study was 20%. As this was obtained from a prospective study of respiratory BAL

investigations, the prevalence in this study should be a reasonable reflection of the prevalence of septic lower airway disease in the veterinary clinic population. CRP is thought to be relatively non-specific and concentrations may be increased in a range of infectious or inflammatory conditions, however, previous studies have shown a more marked increase in infectious disease compared with non-infectious respiratory conditions (Nakamura *et al.*, 2008; Viitanen *et al.*, 2014, 2017). The results in this study show a similar finding, therefore suggesting potential merit in the diagnosis of lower respiratory infections in cases with markedly increased CRP concentrations.

The UK NICE guidelines and British Thoracic Society guidelines for the management of community acquired pneumonia in humans recommend avoidance of prescribing antibiotics in patients with a CRP concentration of < 20 mg/L, and to prescribe antibiotics in those with a CRP of > 100 mg/L due to the high probability of an infectious process at this concentration. This leaves a grey area between 20-100 mg/L where the advice is to monitor patients and delay prescription or perform further diagnostics to confirm or rule out infectious disease (Eccles *et al.*, 2014; Lim *et al.*, 2015). To improve the diagnostic utility of CRP in infectious respiratory disease in dogs, a similar algorithm could be adopted. If infectious disease could be excluded with high probability at < 27 mg/L, then infectious disease could be considered likely with a CRP concentration of > 50 mg/L. This upper limit would allow for cases with significant non-infectious inflammatory disease and allow for cases with potentially milder or resolving infections that may not require antimicrobial therapy, such as the sepsis cases observed in this study with a more moderate increase in CRP concentration. Cases in between these values could be monitored or infectious disease could be confirmed or excluded with further diagnostics. Monitoring of CRP could be

augmented with the use of a critical difference value, which has previously been calculated for canine CRP in one study as 4.85 mg/L (Martínez-Subiela *et al.*, 2003). That is, if the difference between the two measurements whilst monitoring the patient is greater than this value, then the degree of change is likely to be significant, rather than just due to physiologic variation. This study did not assess the use of CRP as a tool for monitoring resolution of infection during antimicrobial therapy. A previous study has shown a benefit of using CRP in monitoring bacterial pneumonia cases to shorten the length of antibiotic treatment courses (Viitanen *et al.*, 2017); however future longitudinal studies could be conducted to assess the benefit of using these proposed CRP guidelines as an initial treatment decision tool, in combination with its use in monitoring resolution of infection in appropriate cases.

Microorganisms were cultured in 4 of the 5 sepsis cases, with various organisms isolated. One case yielded a negative culture despite cytologic confirmation of sepsis, and a CRP concentration of 101.14 and 102 mg/L with the Gentian and Fujifilm assays respectively. This may be due to poor viability of the organisms, recent antimicrobial therapy, or fastidious organisms that did not grow on routine culture. The case diagnosed with neutrophilic inflammation with a CRP concentration of 32 mg/L on the Fujifilm assay yielded no organisms on microbial culture. Although a CRP concentration of 12.32 mg/L was detected with the Gentian assay in the same sample, which would be more typical for a non-infectious case. Microorganisms were isolated in three cases diagnosed with non-infectious disease on cytology, including one case with three different isolates comprising *Pseudomonas* spp. and Beta-haemolytic streptococcus spp., which are not typical commensals of the respiratory tract. These findings could be due to sample contamination

from the environment, skin or upper respiratory tract; or due to incorrect cytologic diagnosis. CRP concentrations in these cases ranged from < 6.8 mg/L to 25 mg/L, which would be less supportive of a diagnosis of lower respiratory infection. A further possible cause for the growth of microorganisms in this case could include in-vitro growth of organisms in stored samples of > 24 hours since sample collection, as detailed in section 5.1.

Prior antibiotic therapy did not appear to affect CRP concentrations in cases diagnosed with septic inflammation. The majority of these cases (n = 4) received antibiotics prior to diagnosis. These findings are similar to previous studies in both human and canine medicine, which reported a similar observation (Bafadhel *et al.*, 2011; Viitanen *et al.*, 2014).

Recent haematology or serum biochemistry blood results were requested upon enrolment in the study, however results were received for only 11 cases (9 of which were diagnostic cases). Patients without blood results were still included in the study due to the low sample numbers and our unexpected difficulties in case recruitment, particularly in comparison with the laboratory BAL caseload obtained from the retrospective study. A neutrophilia was observed in two of the cases diagnosed with septic neutrophilic inflammation, one of which also had an increase in band neutrophils and a monocytosis. These cases had markedly increased CRP concentrations (104 and 102 mg/L Fujifilm) and the correlation between these findings would be expected in many clinical cases of infectious disease. One case with septic inflammation did not show any haematological or biochemical abnormalities, demonstrating a diagnostic advantage in the use of CRP in this case. One case with eosinophilic inflammation also had a moderate peripheral blood eosinophilia, which may suggest a parasitic or allergic disease, or a possible eosinophilic bronchopneumopathy.

In summary, the findings from this study corroborate previous studies showing a marked increase in CRP concentrations with infectious respiratory disease (Nakamura *et al.*, 2008; Viitanen *et al.*, 2014, 2017). Assessment of the diagnostic properties of CRP with ROC curve analysis and supplementary tests showed an optimal sensitivity and specificity to diagnose or exclude infectious lower respiratory disease at 27 mg/L. As discussed earlier, an upper threshold of 50 mg/L could be adopted to ensure more reliable diagnosis of infectious respiratory disease, however the non-specific nature of CRP suggests it is less suited to this purpose. Future prospective clinical trials with respiratory cases utilising decision levels such as these would be required to determine their utility and reliability in discriminating infectious from non-infectious disease, and subsequently use diagnostic guidelines with CRP to determine prescription of antimicrobials.

Future studies in this field may also involve the use of further markers of inflammation, including serum amyloid A (SAA), which has been shown to be as effective, or in some cases more effective than CRP at discriminating infectious disease (Christensen *et al.*, 2014). A further inflammatory marker that may be of interest is procalcitonin, which is used for aiding the diagnosis of infectious conditions or SIRS in human patients (Bafadhel *et al.*, 2011; Van Vugt *et al.*, 2013; Vijayan *et al.*, 2017), and is starting to acquire interest in its use in veterinary medicine (Goggs *et al.*, 2018; Martiny and Goggs, 2019).

5.3 Part 3 – Method Comparison Study

Analytical performance of an assay can be evaluated using pre-determined quality specifications. These quality requirements may be defined in a variety of ways such as those based on clinical outcomes and clinical decision thresholds, requirements based on biologic variation data, or the maximal amount of error as determined by the opinion of experienced clinicians (Westgard, 2008; Harr *et al.*, 2013). No known studies of clinical outcomes or expert opinion-based decision thresholds are available for canine CRP, and so quality requirements for total allowable error and imprecision based on biologic variation data were used in this study.

The calculated within-run and between-run imprecision results for the Fujifilm cCRP assay were all within the desirable allowable imprecision of 12.2% (Kjelgaard-Hansen *et al.*, 2003). A previous study which assessed the Fujifilm assay along with various other methods for measuring canine CRP found an intra-assay precision ranging from 2.01-7.85% for low to high serum concentrations of CRP. The inter-assay precision ranged from 3.55-14.41% (Muñoz Prieto *et al.*, 2017). The assay appeared to be linear upon dilution with small proportional and constant errors. The intercept was -1.5 mg/L with a slope of 1.024, showing good correlation ($r^2 = 0.986$). Due to the small magnitude of the errors the linearity was therefore considered acceptable up to 64 mg/L.

Comparison of the Fujifilm cCRP results with the results from the Gentian cCRP assay showed good correlation ($r = 0.886$). The Deming regression comparison showed a significant proportional bias with a small constant error. The constant error of 1 mg/L is

likely of minimal clinical significance, however the proportional error (slope 1.323 [95% CI 1.27 – 1.37]) has some potential to impact clinical decisions. Using the regression equation: Fujifilm cCRP result = (1.323 x Gentian cCRP result) + 1.0 mg/L the result from the Fujifilm cCRP assay at a proposed clinical decision concentration of 27 mg/L would be 36.7 mg/L, with a bias of 9.7 mg/L or 36%. The combined imprecision and bias exceed the $_{des}TE_a$ for CRP of 29.6%, however the results were within the $_{min}TE_a$ of 44.4% (Kjelgaard-Hansen *et al.*, 2003). A bias of this magnitude, which is beyond the desired acceptable bias of 9.52% (Harr *et al.*, 2013) has been reported for previous point-of-care cCRP assays (Jasensky *et al.*, 2015). The results of the two methods are therefore not interchangeable and the ability to measure serial results with both assays, i.e. measure results using a referral laboratory and in practice machine, is hampered by the significant proportional bias which exceeds the total allowable error for the assay. Assay-specific reference intervals would be needed, and any serial measurements of CRP would need to be performed with the same analyser and method.

Sigma metrics were calculated for the assay after imprecision and inaccuracy were determined as a standardised quantitative measure of overall quality and performance of an assay (Farr and Freeman, 2008). The process focusses on controlling an assay to within a maximum of 6 standard deviations (sigma) of variation (or 3.4 defects per million results) within defined error limits (Westgard and Westgard, 2017). An assay operating at 6 σ is considered excellent or world-class, whereas one operating at 2 σ is considered the minimally acceptable performance. The higher the σ value, the more stable the test and the lower the likelihood that analytical shifts would impact clinical decisions. As such, only large changes in systematic error (or precision) would need to be detected, and less stringent

control procedures would be required (Westgard and Westgard, 2017). The assay was found to be operating at a σ of less than 2 when the $_{des}TE_a$ of 29.6% was set as the defined quality requirement. This is below the limits of stable operation. When the $_{min}TE_a$ of 44.4% is used the assay performs at 3 σ , which is considered acceptable.

The differences in comparative results of the two methods could be resolved by use of a correction factor to reduce the effect of bias on the total error of the assay. The Gentian cCRP assay is most often used on automated chemistry machines, the software of which often has the capacity for correction inputs, whereas the Fujifilm cCRP assay is run on Fujifilm point-of-care analyzers which are designed for non-expert use and correction of results at multiple sites by non-experts would likely be impractical and more prone to error. Therefore, a correction could be applied to the Gentian cCRP assay results most straightforwardly. To investigate the impact of such a correction factor, a proposed correction factor of 30% was added to the results of the Gentian cCRP assay, based on the previously calculated regression slope of 1.323 to reduce the effect of proportional error. This improved the bias of the assay at a CRP concentration of 27 mg/L to 3.4%, based on an improved re-calculated regression equation of $[y = 0.992 x + 1.06 \text{ mg/L}]$ using a Deming regression. Based on these values the TE_{obs} was reduced to 8.9%, well within the $_{des}TE_a$ and $_{min}TE_a$. Therefore, the addition of this adjustment factor would allow for comparison of results due to acceptable levels of agreement between the two methods making results from the two methods interchangeable. A drawback to incorporating such a correction, either within analyzer software or post-analysis, may be a future confusion over the true origin of the results. A further issue may be the lack of agreement between results in EQA schemes, however no currently running EQA schemes include canine CRP assays.

A limitation of this method comparison study is the lack of a true gold standard for the measurement of canine CRP, which limits the interpretation of bias. The reference comparison method used has previously been validated against a human CRP assay calibrated with purified canine CRP (Hillström *et al.*, 2014). The effect of interference from haemolysis, lipaemia, or bilirubin was not assessed in this study, and future studies are required to investigate this further. Previous studies have shown interferences with other CRP assays (Eckersall *et al.*, 1991; Martínez-Subiela and Cerón, 2005), however contrasting results have been reported with others (Parra and Cerón, 2007; Hillström *et al.*, 2014; Hindenberg *et al.*, 2018). The multi-layered film approach of dry chemistry slides, such as those employed in the Fujifilm cCRP assay can mitigate these effects. One further limitation was the variable lower thresholds of detection, requiring standardisation of 50% of the LoQ, which may have caused problems for the statistical analysis and comparison of methods at low concentrations. It should also be noted that the different method types, i.e. wet and dry chemistry, and differing antibodies may account for some of the bias found. Inherent errors in the reference method may also contribute to the bias.

In conclusion, the Fujifilm cCRP assay is a reliable and precise method for the measurement of canine CRP. The results were within minimum quality specifications, however the significant bias indicates that results from the two tests cannot be used interchangeably. The results are therefore not directly comparable and any serial measurements should be interpreted using separately established reference intervals. If implemented a correction factor of +30% would enable the results of the two methods to be used interchangeably.

5.4 Study limitations

Sample recruitment for the prospective CRP BAL study was challenging, with various communications employed to increase engagement with the project and increase sample submission numbers. A frequent issue was the lack of retained spare serum by veterinary practices. During sample recruitment canine BAL or tracheal wash samples were often submitted without any spare serum and were not submitted as part of the study with the relevant consent forms or clinical information. The practices would then be contacted to discuss recruitment of the case into the study. Frequently no spare serum remained to allow case to be included, and many cases also had no blood samples taken as part of the clinical investigations. As a result, sample numbers were lower than expected given the routine laboratory throughput of BAL and retrospective study data, with only small numbers of cases in each disease category. This may limit the statistical power and possible conclusions from the study. However, the findings from this study show a very high statistically significant difference between infectious and non-infectious cases, which is likely to be of diagnostic significance. The large difference in CRP concentration between normal cases and those with infectious disease ensures that conclusions from even small case numbers are likely to be both clinically relevant and statistically significant. Retrospective power calculations with a type 1 error set at 95% (0.05) and using the mean and SD values from septic and non-septic cases, showed that only 5 cases were required per group to show statistical significance with 96.3% power.

Although blood results were requested with sample submission, many submissions did not have associated haematology or biochemistry results. This limited the ability to assess any

associated factors related to the different diagnoses or that may impact on CRP concentration.

Diagnosis of infectious respiratory disease by airway wash cytology and microbial culture is not without faults, with well recognised limitations with each method. Diagnosis via cytology of BAL samples is challenging unless significant changes are observed. The results are dependent on many pre-analytical factors such as the reliability of sample collection and representative samples of the lower respiratory tract. There is also the possibility of sample contamination from the upper airways or oropharynx, which may contaminate the sample both in terms of non-representative cells, and microbial contamination. BALs and tracheal washes are generally non-sterile procedures, which frequently result in sample contamination (Burkhard and Millward, 2010). Other diagnostics such as radiography, computed tomography or endoscopy were not available or routinely provided in the submitted clinical history to use as an additional diagnostic tool.

A further limitation with the method comparison study was the limited amount of surplus serum available for use in the study. This hampered the number of individual tests performed in each experiment and limited the number of replicate analyses that could be performed.

5.5 Conclusions

This study showed a significant difference in the mean concentrations of CRP between infectious and non-infectious lower respiratory tract disease, with the most significant increases found in cases with a cytologic diagnosis of septic neutrophilic inflammation. Cases without cytologic features of sepsis showed only mild or no increases in CRP concentration. The findings from this study indicated that infectious LRT disease could be excluded with a CRP concentration of less than 27 mg/L, using both the automated immunoturbidometric Gentian cCRP assay or the point-of-care quantitative dry chemistry Fujifilm cCRP assay. The properties of CRP as a major acute phase protein and rapid pro-inflammatory marker lends itself for use in discriminating between infectious and non-infectious disease, and the adoption of CRP as an adjunctive decision-making tool in a practice setting may aid in reducing unnecessary prescription of antimicrobials in cases of lower respiratory tract disease.

The prevalence of infectious respiratory disease diagnosed using BAL cytology from the large dataset of cases collected in this study was 16.2%, of which 73% of those cases had a concurrent positive microbial culture. Microorganisms were frequently isolated from cases without a cytologic diagnosis of sepsis, however the clinical significance of these findings is uncertain. Reliable diagnosis of lower respiratory tract infection remains challenging, requiring a multi-modal approach and recognition of the limitations of each diagnostic method. This is the first cross-sectional study of canine respiratory disease cases diagnosed with BAL cytology of this scale.

The comparison of the two CRP methods used in this study showed that the point-of-care Fujifilm cCRP assay would be a precise method for the measurement of CRP concentration. The results were within minimum quality specifications, although the significant bias indicates that results from the two tests should not be used interchangeably without implementation of correction factors, and interpretation of results would require separately established reference intervals.

6. References

- Angus, J. C., Jang, S. S. and Hirsh, D. C. (1997) 'Microbiological study of transtracheal aspirates from dogs with suspected lower respiratory tract disease: 264 cases (1989-1995)', *Journal of the American Veterinary Medical Association*.
- Armengou, L. *et al.* (2008) 'Plasma d-Dimer Concentration in Sick Newborn Foals', *Journal of Veterinary Internal Medicine*, 22(2), pp. 411–417. doi: <https://doi.org/10.1111/j.1939-1676.2008.0050.x>.
- Arnold, J. E. *et al.* (2019) 'ASVCP Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology (version 3.0)', *Veterinary Clinical Pathology*, 48(4), pp. 542–618. doi: 10.1111/vcp.12810.
- Bafadhel, M. *et al.* (2011) 'Procalcitonin and C-reactive protein in hospitalized adult patients with community-acquired pneumonia or exacerbation of asthma or COPD', *Chest*. doi: 10.1378/chest.10-1747.
- Bates, J. *et al.* (2017) 'General practitioner use of a C-reactive protein point-of-care test to help target antibiotic prescribing in patients with acute exacerbations of chronic obstructive pulmonary disease (the PACE study): study protocol for a randomised controlled trial', *Trials*, 18(1), p. 442. doi: 10.1186/s13063-017-2144-8.
- Bathen-Noethen, A. *et al.* (2008) 'Concentrations of Acute-Phase Proteins in Dogs with Steroid Responsive Meningitis-Arteritis', *Journal of Veterinary Internal Medicine*, 22(5), pp. 1149–1156. doi: 10.1111/j.1939-1676.2008.0164.x.
- Bayramli, G. and Ulutas, B. (2008) 'Acute phase protein response in dogs with experimentally induced gastric mucosal injury', *Veterinary Clinical Pathology*, 37(3), pp. 312–316. doi: 10.1111/j.1939-165X.2008.00060.x.
- Biedermann, E., Tipold, A. and Flegel, T. (2016) 'Relapses in dogs with steroid-responsive meningitis-arteritis', *Journal of Small Animal Practice*, 57(2), pp. 91–95. doi: 10.1111/jsap.12418.
- Dabrowski, R., Kostro, K. and Szczubiał, M. (2013) 'Concentrations of C-reactive protein, serum amyloid A, and haptoglobin in uterine arterial and peripheral blood in bitches with pyometra', *Theriogenology*, 80(5), pp. 494–497. doi: 10.1016/j.theriogenology.2013.05.012.
- Buchta, R. *et al.* (1987) 'Modulation of human neutrophil function by C-reactive protein', *European Journal of Biochemistry*, 163(1), pp. 141–146. doi: 10.1111/j.1432-1033.1987.tb10747.x.
- Buchta, R., Pontet, M. and Fridkin, M. (1987) 'Binding of C-reactive protein to human neutrophils', *FEBS Letters*, 211(2), pp. 165–168. doi: 10.1016/0014-5793(87)81429-1.

Burkhard, M. and Millward, L. (2010) 'Respiratory Tract', in Raskin, R. and Meyer, D. (eds) *Canine and Feline Cytology: A Color Atlas and Interpretation Guide, Second Edition*. Second. St. Louis, Missouri: Saunders, pp. 123–170.

Buser, F. C. *et al.* (2019) 'Evaluation of C-reactive protein and its kinetics as a prognostic indicator in canine leptospirosis', *Journal of Small Animal Practice*, 60(8), pp. 477–485. doi: 10.1111/jsap.13004.

Cantos-Barreda, A. *et al.* (2018) 'Relationship between serum anti-Leishmania antibody levels and acute phase proteins in dogs with canine leishmaniosis', *Veterinary Parasitology*, 260, pp. 63–68. doi: 10.1016/j.vetpar.2018.08.010.

Carretón, E. *et al.* (2014) 'Cardiopulmonary and inflammatory biomarkers in the assessment of the severity of canine dirofilariosis', *Veterinary Parasitology*, 206(1–2), pp. 43–47. doi: 10.1016/j.vetpar.2014.08.019.

Caspi, D. *et al.* (1984) 'Isolation and characterization of C-reactive protein from the dog', *Immunology*, 53(2), pp. 307–313.

Caspi, D. *et al.* (1987) 'C-reactive protein in dogs', *American Journal of Veterinary Research*, 48(6), pp. 919–921.

Cerón, J. J. *et al.* (2008) 'A seven-point plan for acute phase protein interpretation in companion animals', *Veterinary Journal*, pp. 6–7. doi: 10.1016/j.tvjl.2007.12.001.

Cerón, J. J., Eckersall, P. D. and Martínez-Subiela, S. (2005) 'Acute phase proteins in dogs and cats: Current knowledge and future perspectives', *Veterinary Clinical Pathology*, 34(2), pp. 85–99. doi: 10.1111/j.1939-165X.2005.tb00019.x.

Chalker, V. J. (2005) 'Canine mycoplasmas', *Research in Veterinary Science*, 79(1), pp. 1–8. doi: 10.1016/J.RVSC.2004.10.002.

Chalker, V. J. *et al.* (no date) 'Mycoplasmas associated with canine infectious respiratory disease'. doi: 10.1099/mic.0.26848-0.

Christensen, M. B. *et al.* (2014) 'Comparison of serum amyloid A and C-reactive protein as diagnostic markers of systemic inflammation in dogs', *Can Vet J*, 55, pp. 161–168.

Clercx, C. *et al.* (2000) 'Eosinophilic bronchopneumopathy in dogs', *Journal of veterinary internal medicine / American College of Veterinary Internal Medicine*. doi: 10.1111/j.1939-1676.2000.tb01168.x.

Conner, J. G. *et al.* (1988) 'Acute phase response in the dog following surgical trauma.', *Research in veterinary science*.

Cray, C., Zaias, J. and Altman, N. H. (2009) 'Acute phase response in animals: a review.', *Comparative medicine*, 59(6), pp. 517–26.

- Curran, M. *et al.* (2020) 'Analysis of the effects of storage temperature and contamination on aerobic bacterial culture results of bronchoalveolar lavage fluid', *Journal of Veterinary Internal Medicine*, 34(1), pp. 160–165. doi: 10.1111/jvim.15686.
- Dabrowski, R., Wawron, W. and Kostro, K. (2007) 'Changes in CRP, SAA and haptoglobin produced in response to ovariohysterectomy in healthy bitches and those with pyometra', *Theriogenology*, 67. doi: 10.1016/j.theriogenology.2006.07.019.
- Dear, J. D. (2014) 'Bacterial pneumonia in dogs and cats', *Veterinary Clinics of North America - Small Animal Practice*, 44(1), pp. 143–159. doi: 10.1016/j.cvsm.2013.09.003.
- Eccles, S. *et al.* (2014) 'Diagnosis and management of community and hospital acquired pneumonia in adults: summary of NICE guidance', *Bmj*, 349(dec03 4), pp. g6722–g6722. doi: 10.1136/bmj.g6722.
- Eckersall, P. D. (2008) 'Proteins, Proteomics, and the Dysproteinemias', in *Clinical Biochemistry of Domestic Animals*. Sixth Edit. doi: 10.1016/B978-0-12-370491-7.00005-2.
- Eckersall, P. D. and Bell, R. (2010) 'Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine', *Veterinary Journal*, 185(1), pp. 23–27. doi: 10.1016/j.tvjl.2010.04.009.
- Eckersall, P. D. and Conner, J. G. (1988) 'Bovine and canine acute phase proteins', *Veterinary Research Communications*, 12(2–3), pp. 169–178. doi: 10.1007/BF00362798.
- Eckersall, P. D., Conner, J. G. and Harvie, J. (1991) 'An immunoturbidimetric assay for canine C-reactive protein', *Vet Res Commun*, 15. doi: 10.1007/BF00497786.
- Eckersall, P. D., Conner, J. G. and Parton, H. (1989) 'An enzyme-linked immunosorbent assay for canine C-reactive protein', *Veterinary Record*, 124(18), pp. 490–491.
- Epstein, S. E., Mellema, M. S. and Hopper, K. (2010) 'Airway microbial culture and susceptibility patterns in dogs and cats with respiratory disease of varying severity', *Journal of Veterinary Emergency and Critical Care*, 20(6), pp. 587–594. doi: 10.1111/j.1476-4431.2010.00587.x.
- Farr, A. J. and Freeman, K. P. (2008) 'Quality control validation, application of sigma metrics, and performance comparison between two biochemistry analyzers in a commercial veterinary laboratory', *Journal of Veterinary Diagnostic Investigation*, 20(5), pp. 536–544. doi: 10.1177/104063870802000502.
- Flatland, B. *et al.* (2018) 'Analytical quality goals-a review', *Vet Clin Pathol*, 47, pp. 527–538. doi: 10.1111/vcp.12649.
- Foster, J. D. *et al.* (2014) 'Serum biomarkers of clinical and cytologic response in dogs with idiopathic immune-mediated polyarthropathy', *Journal of Veterinary Internal Medicine*, 28(3), pp. 905–911. doi: 10.1111/jvim.12351.

- Fransson, B. A., Bergström, A., *et al.* (2007) 'Assessment of three automated assays for C-reactive protein determination in dogs', *American Journal of Veterinary Research*, 68(12), pp. 1281–1286. doi: 10.2460/ajvr.68.12.1281.
- Fransson, B. A., Lagerstedt, A.-S., *et al.* (2007) 'C-reactive protein, tumor necrosis factor α , and interleukin-6 in dogs with pyometra and SIRS', *Journal of Veterinary Emergency and Critical Care*, 17(4), pp. 373–381. doi: 10.1111/j.1476-4431.2006.00203.x.
- Fujise, H. *et al.* (1992) 'Simple isolation of canine C-reactive protein (CRP) by phosphorylcholine (PC) affinity chromatography.', *The Journal of veterinary medical science*, 54(1), pp. 165–7. doi: 10.1292/jvms.54.165.
- Gabay, C. and Kushner, I. (1999) 'Acute-Phase Proteins and Other Systemic Responses to Inflammation', *New England Journal of Medicine*. Edited by F. H. Epstein, 340(6), pp. 448–454. doi: 10.1056/NEJM199902113400607.
- Galezowski, A. M. *et al.* (2010) 'C-reactive protein as a prognostic indicator in dogs with acute abdomen syndrome', *Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, 22(3), pp. 395–401. doi: 10.1177/104063871002200308.
- Goggs, R. *et al.* (2018) 'Plasma procalcitonin concentrations are increased in dogs with sepsis', *Veterinary Record Open*, 5(1), p. e000255. doi: 10.1136/vetreco-2017-000255.
- Griebsch, C. *et al.* (2009) 'C-reactive protein concentration in dogs with primary immune-mediated hemolytic anemia', *Veterinary Clinical Pathology*, 38(4), pp. 421–425. doi: 10.1111/j.1939-165X.2009.00146.x.
- Hakanen, E. *et al.* (2018) 'Urban environment predisposes dogs and their owners to allergic symptoms.', *Scientific reports*, 8(1), p. 1585. doi: 10.1038/s41598-018-19953-3.
- Harr, K. E., Flatland, B., Nabity, M. and Freeman, Kathleen P (2013) 'ASVCP guidelines: Allowable total error guidelines for biochemistry', *Veterinary Clinical Pathology*, 42(4), pp. 424–436. doi: 10.1111/vcp.12101.
- Hartweg, J. *et al.* (2007) 'Stability of soluble adhesion molecules, selectins, and C-reactive protein at various temperatures: Implications for epidemiological and large-scale clinical studies', *Clinical Chemistry*, 53(10), pp. 1858–1860. doi: 10.1373/clinchem.2006.076380.
- Hawkins, E. C. and DeNicola, D. B. (1990) 'Cytologic analysis of tracheal wash specimens and bronchoalveolar lavage fluid in the diagnosis of mycotic infections in dogs.', *Journal of the American Veterinary Medical Association*.
- Hawkins, E. C., DeNicola, D. B. and Plier, M. L. (1995) 'Cytological Analysis of Bronchoalveolar Lavage Fluid in the Diagnosis of Spontaneous Respiratory Tract Disease in Dogs: A Retrospective Study', *Journal of Veterinary Internal Medicine*, 9(6), pp. 386–392. doi: 10.1111/j.1939-1676.1995.tb03298.x.

- Heegaard, P. M. H. *et al.* (1998) 'The Porcine Acute Phase Response to Infection with *Actinobacillus pleuropneumoniae*. Haptoglobin, C-Reactive Protein, Major Acute Phase Protein and Serum Amyloid A Protein Are Sensitive Indicators of Infection', *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*. doi: 10.1016/S0305-0491(97)00362-3.
- Hillstrom, A. *et al.* (2014) 'Validation of a commercially available automated canine-specific immunoturbidimetric method for measuring canine C-reactive protein', *Veterinary Clinical Pathology*, 43(2), pp. 235–243. doi: 10.1111/vcp.12150.
- Hillström, A. *et al.* (2015) 'Validation and application of a canine-specific automated high-sensitivity C-reactive protein assay', *Journal of Veterinary Diagnostic Investigation*, 27(2), pp. 182–190. doi: 10.1177/1040638715575751.
- Hillström, A. *et al.* (2016) 'Measurement of serum C-reactive protein concentration for discriminating between suppurative arthritis and osteoarthritis in dogs', *BMC Veterinary Research*, 12(1), p. 240. doi: 10.1186/s12917-016-0868-4.
- Hindenberg, S. *et al.* (2018) 'Evaluation of a novel quantitative canine species-specific point-of-care assay for C-reactive protein', *BMC Veterinary Research*, 14(1), p. 99. doi: 10.1186/s12917-018-1415-2.
- Hoareau, G. L., Mellema, M. S. and Silverstein, D. C. (2011) 'Indication, management, and outcome of brachycephalic dogs requiring mechanical ventilation', *Journal of Veterinary Emergency and Critical Care*, 21(3), pp. 226–235. doi: 10.1111/j.1476-4431.2011.00635.x.
- Hohenthal, U. *et al.* (2009) 'Utility of C-reactive protein in assessing the disease severity and complications of community-acquired pneumonia', *Clinical Microbiology and Infection*, 15(11), pp. 1026–1032. doi: 10.1111/J.1469-0691.2009.02856.X.
- Huddy, J. R. *et al.* (2016) 'Point-of-care C reactive protein for the diagnosis of lower respiratory tract infection in NHS primary care: A qualitative study of barriers and facilitators to adoption', *BMJ Open*, 6(3). doi: 10.1136/bmjopen-2015-009959.
- Ibraimi, F. *et al.* (2013) 'Preparation of a portable point-of-care in vitro diagnostic system, for quantification of canine C-reactive protein, based on a magnetic two-site immunoassay', in *Analytical and Bioanalytical Chemistry*. Springer-Verlag, pp. 6001–6007. doi: 10.1007/s00216-013-7032-9.
- Israeli, I. *et al.* (2012) 'Serum pepsinogen-A, canine pancreatic lipase immunoreactivity, and C-reactive protein as prognostic markers in dogs with gastric dilatation-volvulus', *Journal of Veterinary Internal Medicine*, 26(4), pp. 920–928. doi: 10.1111/j.1939-1676.2012.00940.x.
- Jabs, W. J. *et al.* (2003) 'The kidney as a second site of human C-reactive protein formation in vivo', *European Journal of Immunology*. doi: 10.1002/immu.200390018.
- Jacobsen, S. *et al.* (2004) 'Dose Dependency and Individual Variability of the Lipopolysaccharide-Induced Bovine Acute Phase Protein Response', *Journal of Dairy Science*. doi: 10.3168/jds.S0022-0302(04)73469-4.

Jacobsen, S. *et al.* (2006) 'Serum amyloid A isoforms in serum and synovial fluid in horses with lipopolysaccharide-induced arthritis', *Veterinary Immunology and Immunopathology*. doi: 10.1016/j.vetimm.2005.10.012.

Jacobsen, S. and Andersen, P. H. (2007) 'The acute phase protein serum amyloid a (SAA) as a marker of inflammation in horses', *Equine Veterinary Education*. doi: 10.2746/095777307X177235.

Jacobsen, S., Thomsen, M. H. and Nanni, S. (2006) 'Concentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease', *American Journal of Veterinary Research*. doi: 10.2460/ajvr.67.10.1738.

Jameson, P. H. *et al.* (1995) 'Comparison of clinical signs, diagnostic findings, organisms isolated, and clinical outcome in dogs with bacterial pneumonia: 93 cases (1986-1991).', *Journal of the American Veterinary Medical Association*.

Jasensky, A. K. *et al.* (2015) 'Evaluation of three different point-of-care tests for quantitative measurement of canine C-reactive protein', *Veterinary Clinical Pathology*, 44(2), pp. 205–214. doi: 10.1111/vcp.12264.

Jensen, A. L., Iversen, L. and Høier, R. (1999) 'Evaluation of analytical performance assisted by total error criteria of a commercial enzyme immunometric assay for canine serum thyrotropin.', *Veterinary clinical pathology / American Society for Veterinary Clinical Pathology*, 28(2), pp. 53–56. doi: 10.1111/j.1939-165X.1999.tb01045.x.

Jensen, A L and Kjølgaard-Hansen, M. (2006) 'Method comparison in the clinical laboratory', *Vet Clin Pathol*, 35. doi: 10.1111/j.1939-165X.2006.tb00131.x.

Jergens, A. E. *et al.* (2003) 'A scoring index for disease activity in canine inflammatory bowel disease', *Journal of Veterinary Internal Medicine*, 17(3), pp. 291–297. doi: 10.1111/j.1939-1676.2003.tb02450.x.

Johnson, L. R. *et al.* (2013) 'Microbiologic and cytologic assessment of bronchoalveolar lavage fluid from dogs with lower respiratory tract infection: 105 cases (2001-2011)', *Journal of Veterinary Internal Medicine*, 27(2), pp. 259–267. doi: 10.1111/jvim.12037.

Kajikawa, T. *et al.* (1999) 'Changes in concentrations of serum amyloid a protein, α 1-acid glycoprotein, haptoglobin, and C-reactive protein in feline sera due to induced inflammation and surgery', *Veterinary Immunology and Immunopathology*, 68(1), pp. 91–98. doi: 10.1016/S0165-2427(99)00012-4.

Kjølgaard-Hansen, M. *et al.* (2003) 'Study on biological variability of five acute-phase reactants in dogs', *Comparative Clinical Pathology*. doi: 10.1007/s00580-003-0477-z.

Kjølgaard-Hansen, M. *et al.* (2006) 'Use of serum C-reactive protein as an early marker of inflammatory activity in canine type II immune-mediated polyarthritis: case report.', *Acta veterinaria Scandinavica*, 48, p. 9. doi: 10.1186/1751-0147-48-9.

Kjelgaard-Hansen, M. *et al.* (2013) 'Canine serum C-reactive protein as a quantitative marker of the inflammatory stimulus of aseptic elective soft tissue surgery', *Veterinary Clinical Pathology*, 42(3), pp. 342–345. doi: 10.1111/vcp.12063.

Kjelgaard-Hansen, M., Jensen, A. L. and Kristensen, A. T. (2003) 'Evaluation of a commercially available human C-reactive protein (CRP) turbidometric immunoassay for determination of canine serum CRP concentration', *Veterinary Clinical Pathology*, 32(2), pp. 81–87. doi: 10.1111/j.1939-165X.2003.tb00319.x.

Kjelgaard-Hansen, M., Kristensen, A. T. and Jensen, A. L. (2003) 'Evaluation of a commercially available enzyme-linked immunosorbent assay (ELISA) for the determination of C-reactive protein in canine serum', *Journal of Veterinary Medicine. A: Physiology, Pathology, Clinical Medicine*, 50(3), pp. 164–168. doi: 10.1046/j.1439-0442.2003.00509.x.

Kjelgaard-Hansen, M., Stadler, M. and Jensen, A. L. (2008) 'Canine serum C-reactive protein detected by means of a near-patient test for human C-reactive protein', *Journal of Small Animal Practice*, 49(6), pp. 282–286. doi: 10.1111/j.1748-5827.2008.00541.x.

Klenner, S., Bauer, N. and Moritz, A. (2010) 'Evaluation of three automated human immunoturbidimetric assays for the detection of C-reactive protein in dogs', *Journal of Veterinary Diagnostic Investigation*, 22(4), pp. 544–552. doi: 10.1177/104063871002200408.

Kocaturk, M. *et al.* (2010) 'Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis', *The Journal of Small Animal Practice*, 51(9), pp. 478–483. doi: 10.1111/j.1748-5827.2010.00965.x.

Kum, C. *et al.* (2013) 'Effects of carprofen and meloxicam on C-reactive protein, ceruloplasmin, and fibrinogen concentrations in dogs undergoing ovariohysterectomy', *American Journal of Veterinary Research*, 74(10), pp. 1267–1273. doi: 10.2460/ajvr.74.10.1267.

Kuribayashi, T. *et al.* (2003) 'Determination of serum C-reactive protein (CRP) in healthy beagle dogs of various ages and pregnant beagle dogs.', *Experimental animals / Japanese Association for Laboratory Animal Science*, 52(May), pp. 387–390. doi: 10.1538/expanim.52.387.

Kushner, I. and Feldmann, G. (1978) 'Control of the acute phase response. Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit.', *Journal of Experimental Medicine*, 148(2), pp. 466–477. doi: 10.1084/jem.148.2.466.

Lampreave, F. *et al.* (1994) 'Characterization of the acute phase serum protein response in pigs.', *Electrophoresis*. doi: 10.1002/elps.1150150195.

Lilliehöök, I. and Tvedten, H. (2003) 'Investigation of hypereosinophilia and potential treatments', *Veterinary Clinics of North America - Small Animal Practice*. doi: 10.1016/S0195-5616(03)00097-4.

- Lim, W. S., Smith, D. L., Wise, M. P., Welham, S. A., Baudouin, S. V, *et al.* (2015) 'BTS guidelines for the management of community acquired pneumonia in adults: update 2009.', *Thorax*, 64 Suppl 3(7), pp. iii1-i55. doi: 10.1136/thoraxjnl-2015-206881.
- Lowrie, M. *et al.* (2009) 'The role of acute phase proteins in diagnosis and management of steroid-responsive meningitis arteritis in dogs', *Veterinary Journal*, 182(1), pp. 125–130. doi: 10.1016/j.tvjl.2008.05.001.
- Mansfield, C. S., James, F. E. and Robertson, I. D. (2008) 'Development of a clinical severity index for dogs with acute pancreatitis', *Journal of the American Veterinary Medical Association*, 233(6), pp. 936–944. doi: 10.2460/javma.233.6.936.
- Martínez-Subiela, S. *et al.* (2002) 'Serum concentrations of acute phase proteins in dogs with leishmaniasis', *Veterinary Record*, 150(8), pp. 241–244. doi: 10.1136/vr.150.8.241.
- Martinez-Subiela, S. *et al.* (2007) 'A time-resolved immunofluorometric assay for porcine C-reactive protein quantification in whole blood', *Luminescence*, 22(3), pp. 171–176. doi: 10.1002/bio.945.
- Martínez-Subiela, S. and Cerón, J. J. (2005) 'Effects of hemolysis, lipemia, hyperbilirrubinemia, and anticoagulants in canine C-reactive protein, serum amyloid A, and ceruloplasmin assays', *Canadian Veterinary Journal*, 46(7), pp. 625–629.
- Martínez-Subiela, S., Ginel, P. J. and Cerón, J. J. (2004) 'Effects of different glucocorticoid treatments on serum acute phase proteins in dogs', *Veterinary Record*. doi: 10.1136/vr.154.26.814.
- Martínez-Subiela, S., Tecles, F. and Cerón, J. J. (2003) 'Critical differences of acute phase proteins in canine serum samples', *Veterinary Journal*, 166(3), pp. 233–237. doi: 10.1016/S1090-0233(02)00315-5.
- Martiny, P. and Goggs, R. (2019) 'Biomarker Guided Diagnosis of Septic Peritonitis in Dogs', *Frontiers in Veterinary Science*, 6, p. 208. doi: 10.3389/fvets.2019.00208.
- McGrotty, Y. L. *et al.* (2004) 'Evaluation of a rapid assay for canine C-reactive protein', *Veterinary Record*, 154, pp. 175–176. doi: 10.1136/vr.154.6.175.
- Méndez, J. C. *et al.* (2014) 'Acute phase response in dogs with *Dirofilaria immitis*', *Veterinary Parasitology*, 204(3–4), pp. 420–425. doi: 10.1016/j.vetpar.2014.05.016.
- Milanović, Z. *et al.* (no date) 'Association of acute *Babesia canis* infection and serum lipid, lipoprotein, and apoprotein concentrations in dogs', *Journal of Veterinary Internal Medicine*, 0(0). doi: 10.1111/jvim.15537.
- Minnaard, M. C. *et al.* (2015) 'The added diagnostic value of five different C-reactive protein point-of-care test devices in detecting pneumonia in primary care: A nested case-control study', *Scandinavian Journal of Clinical and Laboratory Investigation*, 75(4), pp. 291–295. doi: 10.3109/00365513.2015.1006136.

Mischke, R., Waterston, M. and Eckersall, P. D. (2007) 'Changes in C-reactive protein and haptoglobin in dogs with lymphatic neoplasia', *Veterinary Journal*, 174(1), pp. 188–192. doi: 10.1016/j.tvjl.2006.05.018.

Mitchell, K. D. *et al.* (2009) 'Serum acute phase protein concentrations in dogs with autoimmune hemolytic anemia', *Journal of Veterinary Internal Medicine*, 23(3), pp. 585–591. doi: 10.1111/j.1939-1676.2009.0282.x.

Mold, C., Gresham, H. D. and Du Clos, T. W. (2001) 'Serum amyloid P component and C-reactive protein mediate phagocytosis through murine Fc gamma Rs.', *Journal of immunology (Baltimore, Md. : 1950)*. doi: 10.4049/JIMMUNOL.166.2.1200.

Mukorera, V. *et al.* (2011) 'Serum C-reactive protein concentration in benign and malignant canine spirocercosis', *Journal of Veterinary Internal Medicine*, 25(4), pp. 963–966. doi: 10.1111/j.1939-1676.2011.0733.x.

Muñoz Prieto, A. *et al.* (2017) 'Use of heterologous immunoassays for quantification of serum proteins: The case of canine C-reactive protein', *PLoS ONE*. Edited by S. V. Pizzo, 12(2), p. e0172188. doi: 10.1371/journal.pone.0172188.

Murata, H., Shimada, N. and Yoshioka, M. (2004) 'Current research on acute phase proteins in veterinary diagnosis: an overview', *The Veterinary Journal*, 168(1), pp. 28–40. doi: 10.1016/S1090-0233(03)00119-9.

Mylonakis, M. E. *et al.* (2011) 'Serum acute phase proteins as clinical phase indicators and outcome predictors in naturally occurring canine monocytic ehrlichiosis', *Journal of Veterinary Internal Medicine*, 25(4), pp. 811–817. doi: 10.1111/j.1939-1676.2011.0728.x.

Mylonakis, M. E. *et al.* (2012) 'Serum acute phase proteins in dogs with symptomatic esophageal spirocercosis', *Veterinary Parasitology*, 190(1–2), pp. 191–195. doi: 10.1016/j.vetpar.2012.05.013.

Nakamura, M. *et al.* (2008) 'C-reactive protein concentration in dogs with various diseases', *J Vet Med Sci*, 70. doi: 10.1292/jvms.70.127.

National Institute for Health and Care Excellence (2014) *Pneumonia in adults : diagnosis and management*.

Nevalainen, D. *et al.* (2000) 'Evaluating Laboratory Performance on Quality Indicators With the Six Sigma Scale', *Arch Pathol Lab Med*, 124, p. 4.

Nielsen, L *et al.* (2007) 'Serum C-reactive protein concentration as an indicator of remission status in dogs with multicentric lymphoma', *J Vet Intern Med*, 21. doi: 10.1111/j.1939-1676.2007.tb01943.x.

Nikvand, A. A. *et al.* (2019) 'Clinical, hematologic, hemostatic, and serum biochemical findings related to survival in Arabian horses with colic', *Veterinary Clinical Pathology*, 48(3), pp. 441–448. doi: <https://doi.org/10.1111/vcp.12779>.

Nivy, R. *et al.* (2014) 'Serum acute phase protein concentrations in dogs with spirocerosis and their association with esophageal neoplasia - A prospective cohort study', *Veterinary Parasitology*, 203(1–2), pp. 153–159. doi: 10.1016/j.vetpar.2014.02.001.

Office for National Statistics, Open Geography Portal. (2011) *ONSPD Centroids Lite*, *ONSPD Centroids Lite*. Available at: <https://geoportal.statistics.gov.uk/datasets/onspd-centroids-lite?geometry=-29.132,51.076,24.261,59.762> (Accessed: February 2019).

Otabe, K. *et al.* (1998) 'Physiological levels of C-reactive protein in normal canine sera.', *Veterinary research communications*, 22(2), pp. 77–85.

Otabe, K. *et al.* (2000) 'C-reactive protein (CRP) measurement in canine serum following experimentally-induced acute gastric mucosal injury', *Laboratory Animals*, 34(4), pp. 434–438. doi: 10.1258/002367700780387679.

Packer, R. M. A. *et al.* (2019) 'Great expectations, inconvenient truths, and the paradoxes of the dog-owner relationship for owners of brachycephalic dogs', *PloS One*, 14(7), p. e0219918. doi: 10.1371/journal.pone.0219918.

Paltrinieri, S. (2008) 'The feline acute phase reaction', *Veterinary Journal*, 177(1), pp. 26–35. doi: 10.1016/j.tvjl.2007.06.005.

Pardo-Marin, L. *et al.* (2020) 'Comparison of acute phase proteins in different clinical classification systems for canine leishmaniosis', *Veterinary Immunology and Immunopathology*, 219, p. 109958. doi: 10.1016/j.vetimm.2019.109958.

Parra, M. D. *et al.* (2005) 'Use of a time-resolved immunofluorometric assay for determination of canine C-reactive protein concentrations in whole blood', *American Journal of Veterinary Research*, 66(1), pp. 62–66. doi: 10.2460/ajvr.2005.66.62.

Parra, M. D. *et al.* (2006) 'Analytical and clinical validation of a time-resolved immunofluorometric assay (TR-IFMA) for canine C-reactive protein in serum', *Veterinary Research Communications*, 30(2), pp. 113–126. doi: 10.1007/s11259-006-3201-z.

Parra, M. D. and Cerón, J. J. (2007) 'Effects of haemolysis, lipaemia and bilirubinaemia in canine C-reactive protein and haptoglobin determination by time-resolved fluorometry: short communication.', *Acta veterinaria Hungarica*, 55(3), pp. 295–9. doi: 10.1556/AVet.55.2007.3.3.

Peeters, D. E. *et al.* (2000) 'Quantitative bacterial cultures and cytological examination of bronchoalveolar lavage specimens in dogs', *Journal of veterinary internal medicine / American College of Veterinary Internal Medicine*, 14(5), pp. 534–541. doi: 10.1111/j.1939-1676.2000.tb02272.x.

Petersen, H. H., Nielsen, J. P. and Heegaard, P. M. H. (2004) 'Application of acute phase protein measurements in veterinary clinical chemistry', *Veterinary Research*. EDP Sciences, pp. 163–187. doi: 10.1051/vetres:2004002.

- Piñeiro, M. *et al.* (2018) 'A new automated turbidimetric immunoassay for the measurement of canine C-reactive protein', *Veterinary Clinical Pathology*. doi: 10.1111/vcp.12576.
- Planellas, M. *et al.* (2012) 'Evaluation of C-reactive protein, haptoglobin and cardiac troponin 1 levels in brachycephalic dogs with upper airway obstructive syndrome', *BMC veterinary research*, 8, p. 152. doi: 10.1186/1746-6148-8-152.
- Plickert, H. D. *et al.* (2011) 'Evaluation of a point-of-care test for canine C-reactive protein', *Veterinary Clinical Pathology*, 40(3), pp. 384–388. doi: 10.1111/j.1939-165X.2011.00339.x.
- Priestnall, S. L. *et al.* (2014) 'New and Emerging Pathogens in Canine Infectious Respiratory Disease', *Veterinary Pathology*, 51(2), pp. 492–504. doi: 10.1177/0300985813511130.
- Radhakrishnan, A. *et al.* (2007) 'Community-acquired infectious pneumonia in puppies: 65 cases (1993–2002)', *Journal of the American Veterinary Medical Association*. doi: 10.2460/javma.230.10.1493.
- Raila, J., Schweigert, F. J. and Kohn, B. (2011) 'C-reactive protein concentrations in serum of dogs with naturally occurring renal disease', *Journal of Veterinary Diagnostic Investigation*, 23(4), pp. 710–715. doi: 10.1177/1040638711407896.
- Rawlinson, J. E. *et al.* (2011) 'Association of periodontal disease with systemic health indices in dogs and the systemic response to treatment of periodontal disease', *Journal of the American Veterinary Medical Association*, 238(5), pp. 601–609. doi: 10.2460/javma.238.5.601.
- Rebar, A. H., DeNicola, D. B. and Muggenburg, B. A. (1980) 'Bronchopulmonary lavage cytology in the dog: Normal findings', *Veterinary Pathology*. doi: 10.1177/030098588001700303.
- Reimann, M. J. *et al.* (2016) 'Increased serum C-reactive protein concentrations in dogs with congestive heart failure due to myxomatous mitral valve disease', *Veterinary Journal (London, England: 1997)*, 209, pp. 113–118. doi: 10.1016/j.tvjl.2015.12.006.
- Riley, R. F. and Coleman, M. K. (1970) 'Isolation of C-reactive proteins of man monkey, rabbit and dog by affinity chromatography on phosphorylated cellulose', *Clinica Chimica Acta*, 30(2), pp. 483–496. doi: 10.1016/0009-8981(70)90141-5.
- Rycroft, A. N., Tsounakou, E. and Chalker, V. (2007) 'Serological evidence of Mycoplasma cynos infection in canine infectious respiratory disease', *Veterinary Microbiology*. doi: 10.1016/j.vetmic.2006.11.011.
- Salem, N. Y. *et al.* (2020) 'Canine demodicosis: Hematological and biochemical alterations', *Veterinary World*, 13(1), pp. 68–72. doi: 10.14202/vetworld.2020.68-72.
- Schmidt, E. M. S. *et al.* (2016) 'Acute phase proteins in dogs naturally infected with the Giant Kidney Worm (Dioctophyme renale)', *Veterinary Clinical Pathology*, 45(4), pp. 657–664. doi: 10.1111/vcp.12400.

- Selting, K. A. *et al.* (2015) 'Serum thymidine kinase 1 and C-reactive protein as biomarkers for screening clinically healthy dogs for occult disease', *Veterinary and Comparative Oncology*, 13(4), pp. 373–384. doi: 10.1111/vco.12052.
- Selting, K. A. *et al.* (2016) 'Thymidine Kinase Type 1 and C-Reactive Protein Concentrations in Dogs with Spontaneously Occurring Cancer', *Journal of veterinary internal medicine*, 30(4), pp. 1159–1166. doi: 10.1111/jvim.13954.
- Seo, K. W. *et al.* (2012) 'C-reactive protein as an indicator of inflammatory responses to experimentally induced cystitis in dogs', *Journal of Veterinary Science*, 13(2), pp. 179–185. doi: 10.4142/jvs.2012.13.2.179.
- Severo, J. S. *et al.* (2018) 'Evaluation of C-reactive protein as an inflammatory marker of pemphigus foliaceus and superficial pyoderma in dogs', *Veterinary Dermatology*, 29(2), pp. 128-e51. doi: 10.1111/vde.12510.
- Shaddock, E. J. (2016) 'How and when to use common biomarkers in community-acquired pneumonia', *Pneumonia*, 8(1), p. 17. doi: 10.1186/s41479-016-0017-7.
- Shrive, a K. *et al.* (1996) 'Three dimensional structure of human C-reactive protein.', *Nature structural biology*. doi: 10.1038/nsb0496-346.
- Smith, R. P. *et al.* (1995) 'C-Reactive Protein: A Clinical Marker in Community-Acquired Pneumonia', *Chest*, 108(5), pp. 1288–1291. doi: 10.1378/CHEST.108.5.1288.
- Smith, R. P. and Lipworth, B. J. (1995) 'C-reactive protein in simple community-acquired pneumonia', *Chest*, 107(4), pp. 1028–1031. doi: 10.1378/chest.107.4.1028.
- Tagata, K. *et al.* (1996) 'Quantitative capillary reversed passive latex agglutination test for C-reactive protein (CRP) in the dog', *Veterinary Research Communications*, 20(1), pp. 21–30. doi: 10.1007/BF00346573.
- Thompson, D., Pepys, M. B. and Wood, S. P. (1999) 'The physiological structure of human C-reactive protein and its complex with phosphocholine', *Structure*, 7(2), pp. 169–177. doi: 10.1016/S0969-2126(99)80023-9.
- Tillett, W. S. and Francis, T. (1930) 'SEROLOGICAL REACTIONS IN PNEUMONIA WITH A NON-PROTEIN SOMATIC FRACTION OF PNEUMOCOCCUS.', *The Journal of experimental medicine*, 52(4), pp. 561–71. doi: 10.1084/jem.52.4.561.
- Torrente, C. *et al.* (2015) 'Plasma iron, C-reactive protein, albumin, and plasma fibrinogen concentrations in dogs with systemic inflammatory response syndrome', *Journal of Veterinary Emergency and Critical Care*, 25(5), pp. 611–619. doi: 10.1111/vec.12340.
- Uhlir, C. M. and Whitehead, A. S. (1999) 'Serum amyloid A, the major vertebrate acute-phase reactant', *European Journal of Biochemistry*, 265(2), pp. 501–523. doi: 10.1046/j.1432-1327.1999.00657.x.

- Ulutas, B. *et al.* (2005) 'Serum concentration of some acute phase proteins in naturally occurring canine babesiosis: A preliminary study', *Veterinary Clinical Pathology*, 34(2), pp. 144–147. doi: 10.1111/j.1939-165X.2005.tb00028.x.
- Vail, D. M., Mahler, P. A. and Soergel, S. A. (1995) 'Differential cell analysis and phenotypic subtyping of lymphocytes in bronchoalveolar lavage fluid from clinically normal dogs.', *American Journal of Veterinary Research*.
- Van Vugt, S. F. *et al.* (2013) 'Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients presenting to primary care with acute cough: Diagnostic study', *BMJ (Online)*, 346(7909). doi: 10.1136/bmj.f2450.
- Viitanen, S. J. *et al.* (2014) 'Serum C-reactive protein as a diagnostic biomarker in dogs with bacterial respiratory diseases', *Journal of Veterinary Internal Medicine*, 28(1), pp. 84–91. doi: 10.1111/jvim.12262.
- Viitanen, S. J. *et al.* (2017) 'The Utility of Acute-Phase Proteins in the Assessment of Treatment Response in Dogs With Bacterial Pneumonia', *Journal of Veterinary Internal Medicine*, 31(1), pp. 124–133. doi: 10.1111/jvim.14631.
- Vijayan, A. L. *et al.* (2017) 'Procalcitonin: a promising diagnostic marker for sepsis and antibiotic therapy', *Journal of Intensive Care*, 5. doi: 10.1186/s40560-017-0246-8.
- Volanakis, J. E. (2001) 'Human C-reactive protein: expression, structure, and function', *Molecular Immunology*, 38(2), pp. 189–197. doi: 10.1016/S0161-5890(01)00042-6.
- Van Vugt, S. F. *et al.* (2013) 'Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients presenting to primary care with acute cough: Diagnostic study', *BMJ (Online)*, 346(7909). doi: 10.1136/bmj.f2450.
- Westgard, J. O. (1995) 'A method evaluation decision chart (MEDx chart) for judging method performance', *Clinical Laboratory Science: Journal of the American Society for Medical Technology*, 8(5), pp. 277–283.
- Westgard, J. O. (2006) *Six Sigma Quality Design & Control*. 2nd edn. Madison, WI: Westgard QC.
- Westgard, J. O. (2008) *Basic Method Validation*. 3rd edn. Madison, WI: Westgard QC.
- Westgard, J. O. and Westgard, S. A. (2017) 'Six Sigma Quality Management System and Design of Risk-based Statistical Quality Control', *Clinics in Laboratory Medicine*. Elsevier, pp. 85–96. doi: 10.1016/j.cll.2016.09.008.
- Wong, V. M. *et al.* (2011) 'Serum C-reactive protein concentrations in healthy Miniature Schnauzer dogs', *Veterinary Clinical Pathology*, 40(3), pp. 380–383. doi: 10.1111/j.1939-165X.2011.00336.x.

Worksheets (Downloads) - Westgard (no date). Available at: <https://www.westgard.com/downloads/worksheets-downloads.html> (Accessed: 2 March 2021).

Yamamoto, S. *et al.* (1992) 'Isolation of canine C-reactive protein and characterization of its properties.', *Veterinary immunology and immunopathology*, 30(4), pp. 329–39.

Yamamoto, S., Miyaji, S., *et al.* (1993) 'Canine C-reactive protein (CRP) does not share common antigenicity with human CRP', *Veterinary Research Communications*, 17(4), pp. 259–266. doi: 10.1007/BF01839216.

Yamamoto, S., Shida, T., *et al.* (1993) 'Changes in serum C-reactive protein levels in dogs with various disorders and surgical traumas', *Veterinary Research Communications*, 17(2), pp. 85–93. doi: 10.1007/BF01839236.

Yamamoto, S. *et al.* (1994) 'Determination of C-reactive protein in serum and plasma from healthy dogs and dogs with pneumonia by ELISA and slide reversed passive latex agglutination test.', *The Veterinary quarterly*, 16(2), pp. 74–77. doi: 10.1080/01652176.1994.9694422.

Yamamoto, S *et al.* (1994) 'Serum C-reactive protein and immune responses in dogs inoculated with *Bordetella bronchiseptica* (phase I cells)', *Veterinary Research Communications*, 18(5), pp. 347–357. doi: 10.1007/BF01839285.

Yamashita, K. *et al.* (1994) 'Canine acute phase response: relationship between serum cytokine activity and acute phase protein in dogs', *Journal of Veterinary Medical Science*.

Zabrecky, K. A. *et al.* (2015) 'Plasma C-Reactive Protein and Haptoglobin Concentrations in Critically Ill Neonatal Foals', *Journal of Veterinary Internal Medicine*, 29(2), pp. 673–677. doi: <https://doi.org/10.1111/jvim.12568>.

Zhong, W. *et al.* (1998) 'Effect of Human C-Reactive Protein on Chemokine and Chemotactic Factor-Induced Neutrophil Chemotaxis and Signaling', *The Journal of Immunology*, 161(5), p. 2533 LP – 2540.

Appendices

Appendix 1: Consent form



T: 01379 854180
F: 01379 852424
Email: email@finnpathologists.com

Title of the study: C-reactive protein – a tool for reducing unnecessary antibiotic use?

Researchers:

Guy Davies (Finn Pathologists/University of Nottingham): guy.davies@nottingham.ac.uk
Rand Wilson (Finn Pathologists): Rand.Wilson@finnpathologists.com
Peter Graham (University of Nottingham): peter.graham@nottingham.ac.uk

Purpose of the study:

This study will determine whether C-reactive protein (CRP), a protein that can be measured in a blood sample, can help determine whether it is appropriate to use antibiotics to treat dogs with conditions affecting their breathing such as chest infections. Dogs that have breathing problems that might have a chest infection, and that have a clinical procedure called a bronchoalveolar-lavage (BAL), are eligible for inclusion in this study. All procedures undertaken as part of this study would be carried out as part of the normal management of the dog's condition. No additional procedures over and above what is recommended to investigate and manage the condition will be undertaken. Blood that has been left over after the recommended tests have been performed on it will be stored and additional project related tests including CRP will be performed on it. Comparing the results of the extra CRP test with the results of the BAL procedure will help determine how we can use the CRP test to decide on antibiotic therapy.

Consent:

This consent form is a formal way of indicating that you agree to participate in this study and that you understand that any information collected by the researchers:

- will be used for a research study
- may be written in a report for publication
- may be presented at research conferences or meetings
- will be anonymised and treated confidentially
- will only be accessed by research colleagues or examiners
- that you can request to see any information written down/kept during the process of data collection.

	Please tick
I understand that participation in this study is voluntary and that I may leave the study at any time (without needing to provide reasons for doing so)	
I agree that information I give during the study can be used in a report, a published paper or a conference or meeting presentation.	
I understand that the study is being conducted for the purposes of research.	
I understand that I can request to see a summary of the findings, and I can also request to see any notes made during the process of my data collection.	

If you have any queries regarding this study, please speak to the researcher directly or contact them via e-mail or phone (details above).

If applicable, please tick here if you would like to receive a summary of the study results, and indicate how you would like to be contacted

.....

Participant

Name:..... Signature:.....

Date:.....

Thank you very much for participating in this study.

PLEASE SEND THIS FORM TO THE LAB WITH THE SAMPLE SUBMISSION FORM

Appendix Figure 1 – Client consent form

Appendix 2: Laboratory Submission form



T: 01379 854180
F: 01379 852424
Email: email@finnpathologists.com

CRP TRIAL – Submission Form

I am submitting this case to:

Finn ☐

Axiom ☐

Greendale ☐

Submitting Vet.....

Practice Address and Postcode:

Owner

Animal

Species CANINE

Breed

Age

Sex M / F

Neutered Y / N

Date Sample Taken:

PRACTICE TO COMPLETE:			LABORATORY USE ONLY:		
Please ensure all of the following is included:			Tests required: [Please tick]		
Please tick as completed			Finn	CYTCU	☐
			Axiom	WASME + VCUL2	☐
			Greendale	BAL	☐
			[Please tick]		
BAL Sample			Samples Received:		☐
EDTA			History received:		☐
Plain			<i>Please call for history if not received</i>		☐
Smears			Consent form received:		☐
Serum blood sample			<i>Please call for consent form if not received</i>		☐
Clinical history			Pre-anaesthetic results received:		☐
Owner consent form			<i>(if client has ticked to confirm run in-house)</i>		☐
Pre-anaesthetic results (if completed in-house)			CRP Results:		
			Olympus	☐	
			Fuji	☐	

Please attach your Robovet submission to the back of this form

Appendix Figure 2 – Laboratory submission form

Client information sheet



Clinical Study: C-reactive protein (CRP): A tool for reducing unnecessary antibiotic use?

A collaborative study between CVS Group Practices and Laboratories, and the University of Nottingham.

We would like to invite you and your pet to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. If you have any questions do not hesitate to contact researcher Guy Davies via email (guy.davies@nottingham.ac.uk).

Purpose of the study

The aim of this project is to discover whether C-reactive protein (CRP) could be used as a means to help guide antibiotic use and duration of treatment in dogs.

CRP is a protein in blood that is produced by the liver as part of the immune response to an infection.

We are investigating the measurement of this protein in diagnosing chest infections in dogs, as well as treatment decision making, in particular whether CRP result can help vets decide whether to prescribe antibiotics in the future. The use of CRP in suspected chest infections in people has already helped to reduce unnecessary antibiotic use and reduce the length of antibiotic courses in some patients.

This study will aim to see if CRP could be used in a similar way in veterinary medicine.

What happens if I take part?

We propose to measure CRP concentration in a blood sample from your dog, and compare these results to cytology and bacterial culture samples from respiratory tract washes (also called a Bronchoalveolar lavage) – a common procedure to investigate respiratory problems.

Many cases with respiratory disease will have already had, or be due to have a blood sample in the course of the investigation into their condition, especially if they may need to undergo a general anaesthetic for further investigations. Any left-over blood sample can be used to measure CRP so it will not be necessary to take an extra blood sample for this study.

All procedures undertaken as part of this study would be carried out as part of the normal clinical management of the dog's condition. No additional procedures over and above what is recommended to investigate and manage the condition will be undertaken.

Results of the respiratory wash cytology, bacterial culture and blood tests will then be sent to the lab to be analysed by a pathologist as usual. The normal test results can be analysed by CVS laboratories and reported back to your vet, who can then discuss the results with you.

The results of the CRP analysis would not be available straight away, and so would not impact the management or treatment of your pet, but a summary of the results could be provided on completion of the study if requested.

Inclusion criteria

- Dogs with respiratory disease where bacterial pneumonia is a possible cause
- Cases where a blood sample has been taken or due to be taken within 24 hours of a bronchoalveolar lavage (respiratory tract wash)
- Prior antibiotic or other treatment is not a factor for exclusion.

The test for CRP will be free of charge for patients included in the study, but the other diagnostic tests and any treatments deemed necessary by your vet would be charged as normal by your practice.

Will taking part in the study be kept confidential?

In order to maintain participant confidentiality, once the results from the samples are reported back to your vet from the lab in the usual way, participants will then be assigned participant numbers to anonymise the data. We will follow ethical and legal practice and all information will be handled in confidence. The project was reviewed by the University Of Nottingham ethical review committee. If you join the study, some parts of the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly.

All information which is collected during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you will have your name and any identifying information removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Inclusion of dogs in this study is entirely voluntary, and if your dog is not included in this study it will not in any way affect the care your dog receives from your practice.

If you have any questions then please speak to your vet directly, or contact any of the researchers involved in the project:

Guy Davies (University Of Nottingham/Finn Pathologists): guy.davies@nottingham.ac.uk Peter Graham (University Of Nottingham): peter.graham@nottingham.ac.uk Rand Wilson (Finn Pathologists): rand.wilson@finnpathologists.com

Practice information sheet



Group plc
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Clinical Study: C-reactive protein (CRP): A tool for reducing unnecessary antibiotic use?

A collaborative study between CVS Group Practices and Laboratories, and the University of Nottingham.

We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. If you have any questions do not hesitate to contact the researchers on the following emails: Guy Davies (guy.davies@nottingham.ac.uk); Peter Graham (peter.graham@nottingham.ac.uk); Rand Wilson (rand.wilson@finnpathologists.com).

Study outline/summary

The aim of this project is to discover whether C-reactive protein (CRP) could be used as a means to help guide antibiotic use in patients, as well as potentially aid in the diagnosis of bacterial pneumonia in dogs

We propose to measure CRP concentrations from serum and compare these results to microbiological culture and cytology from respiratory washes of affected cases. These results can be analysed by CVS laboratories and reported to the clinician via the normal route.

The results of the CRP analysis would not be available straight away, but a summary of the results could be provided on completion of the study if requested.

Inclusion criteria and Samples to be taken

- Dogs with respiratory disease where bacterial pneumonia is a differential diagnosis
- Cases where a blood sample has been taken or due to be taken within 24 hours of a bronchoalveolar lavage
 - Spare serum can be sent where pre-GA/comprehensive in-house blood tests are analysed, but please send the in-house results with the samples.
 - Otherwise the sample could be run on serum sent to the lab for external analysis, if the BAL is performed within 24 hours of sample being taken.
- Lavage fluid needs to be taken in EDTA and plain tubes, then sent for microbiological culture and cytology to CVS laboratories
- Prior antibiotic treatment is not a factor for exclusion.

A checklist has been provided to ensure all the relevant samples and information are sent to the lab. A consent form is also available for clients to sign to enrol in the study. Clients cannot be included in the study without completion of this consent form.

Please send your samples to your lab (Finn/Axiom/Greendale) in the normal way with the additional checklist completed by the practice, and the consent form completed by the client. Any samples sent to Axiom or Greendale via your courier will be forwarded to Finn to ensure continuity of analysis and reporting.

Purpose of study

The aim of this project is to discover whether CRP could be used as a means to help guide antibiotic use in patients, as well as potentially aid in the diagnosis of bacterial pneumonia in dogs. We are using cases of lower respiratory disease, in particular bacterial pneumonia as a model for this study. The use of CRP in helping guide clinicians on appropriate antibiotic use in these cases is already established in human medicine, as recommended by the NICE guideline for pneumonia published in 2014.

The study will aim to characterise the use of CRP in differentiating cases of canine bacterial pneumonia from other lower respiratory tract diseases, and therefore whether its use can guide prescription of antibiotics for these patients. We will also aim to evaluate the use of CRP as a relatively simple and inexpensive point-of-care biochemical test in these cases where often other diagnostics may be too costly or potentially risky to perform.

Will taking part in the study be kept confidential?

In order to maintain participant confidentiality, after the results from the samples are reported back from the lab in the usual way, participants (clients and practices) will then be assigned participant numbers to anonymise the data. We will follow ethical and legal practice and all information about your practice and clients will be handled in confidence. The project was reviewed by the University of Nottingham ethical review committee. Some parts of the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly.

All information which is collected during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any identifying information about your practice or clients will be removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

For further information please contact any of the researchers via the above email addresses.

Further information

CRP is an acute phase protein, part of the innate immune system, and produced by the liver in response to inflammatory stimuli. This is a non-specific response but has the benefit of a rapid increase in response to the stimulus (4-6 hours), a peak level within 48 hours, and return to normal within 1-2 weeks upon resolution of the inflammatory process. Other acute phase proteins you may have heard of include: serum amyloid A (SAA), Haptoglobin, or alpha1-acid glycoprotein (AGP). These proteins have been shown to increase in many infectious, inflammatory or neoplastic conditions, and so their use is not specific. However, they are rarely increased in non-infectious cases of respiratory disease, so they have promising use for differentiating patients that may require antimicrobial therapy, as well as being useful for monitoring response to treatment.

Appendix 5: Retrospective study - extraction of BAL cases from laboratory databases.

Further analysis was required to identify BAL cases from the retrospective data collected from the CVS laboratory databases. A Microsoft Excel formula was used to extract potential airway wash cases from the data. The formula searched for common terms for respiratory washes which are commonly stated in the 'Sample received' or 'History' sections of cytology reports. The formula used was:

`=SUMPRODUCT(--ISNUMBER(SEARCH(respterms,K3)))>0`

With the following search terms grouped 'respterms' within the spreadsheet:

BAL, BALF, TTW, TW, Tracheal, tracheal, bronch, alveol, lavage, airway

An example cytology report obtained from the database:

SAMPLE RECEIVED: BAL fluid (sample 1). MACROSCOPIC APPEARANCE: Colorless, clear, thin, no clots seen.

MICROSCOPIC DESCRIPTION: Direct smear and cytocentrifuged preparation are examined. The direct smear reveals slightly increased cellularity of nucleated cells and very rare erythrocytes. Differential count (approximate give poor preservation of nucleated cells) yields 80% neutrophils, 18% large mononuclear cells, 1% small lymphocytes and 1% eosinophils. Moderate numbers of columnar ciliated respiratory epithelial cells seen. Small amounts of mucous and ruptured cells are present in the background. No infectious agents or overtly neoplastic cells seen. CYTOLOGIC INTERPRETATION: Neutrophilic inflammation. COMMENTS: Although no infectious agents were observed in the sample, an infectious aetiology cannot be cytologically ruled out. Culture and sensitivity results will follow.

A formula was then subsequently used to extract the cytologic interpretation from each of the case reports to enable further analysis, as no specific diagnosis or interpretation fields are recorded in the database. The formula used was:

`=MID(P45,SEARCH("Interpretation",P45), SEARCH("COMMENT",P45,SEARCH("Interpretation",P45)) - SEARCH("Interpretation",P45))`

The result of the formula for the above example would be:

INTERPRETATION: Neutrophilic inflammation.

The extracted interpretation could then be used to categorise cases more easily for further analysis.