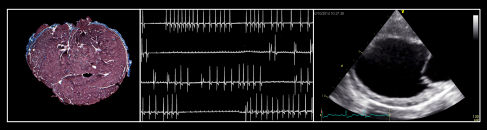

Scientific Forum Exploring Causes of DCM in Dogs, Sept. 29, 2020

Prevalence of Selected Cardiotropic Pathogens in the Myocardium of Adult Dogs with Unexplained Myocardial and Rhythm Disorders

Dott. R.A. Santilli Med.Vet.,PhD, D.E.C.V.I.M.-C.A. (Cardiology)
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European Heart Journal (2013) 34, 2636–2648
doi:10.1093/eurheartj/eht210

Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases

Definitions

Myocarditis (WHO /ISFC[1]):

Inflammatory disease of the myocardium diagnosed by established histological, immunological and immunohistochemical criteria**.*

**N.B. established histological Dallas criteria[12] defined as follows: 'histological evidence of inflammatory infiltrates within the myocardium associated with myocyte degeneration and necrosis of non-ischaemic origin'[12];*

***N.B. unspecified immunohistochemical criteria[1], we propose an abnormal inflammatory infiltrate to be defined as follows: '≥ 14 leucocytes/mm^2 including up to 4 monocytes/mm^2 with the presence of CD 3 positive T-lymphocytes ≥ 7 cells/mm^2'.[13,18,19]*

Inflammatory Cardiomyopathy (WHO /ISFC[1]):

Myocarditis in association with cardiac dysfunction.

N.B. Inflammatory cardiomyopathy, involved in the pathogenesis of DCM, includes idiopathic, autoimmune and infectious subtypes.[1]

Dilated Cardiomyopathy (ESC[14]; WHO /ISFC[1]):

DCM is a clinical diagnosis characterized by dilation and impaired contraction of the left or both ventricles that is not explained by abnormal loading conditions or coronary artery disease.

N.B. DCM includes idiopathic, familial/genetic, viral and/or immune, alcoholic/toxic subtypes.[1]

European Heart Journal (2013) 34, 2636–2648
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Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases

Inflammatory Cardiomyopathy (WHO /ISFC[1]):

Myocarditis in association with cardiac dysfunction.

N.B. Inflammatory cardiomyopathy, involved in the pathogenesis of DCM, includes idiopathic, autoimmune and infectious subtypes.[1]

DCM$_i$

Journal of the American College of Cardiology
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ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.09.074

STATE-OF-THE-ART PAPER

Update on Myocarditis

Ingrid Kindermann, MD,* Christine Barth,* Felix Mahfoud, MD,* Christian Ukena, MD,* Matthias Lenski, MD,* Ali Yilmaz, MD,† Karin Klingel, MD,‡ Reinhard Kandolf, MD,‡ Udo Sechtem, MD,† Leslie T. Cooper, MD,§ Michael Böhm, MD*

Homburg/Saar, Stuttgart, and Tübingen, Germany; and Rochester, Minnesota

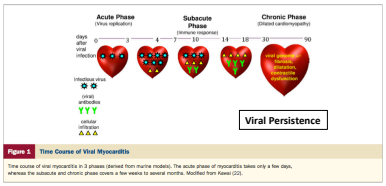


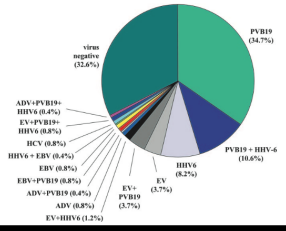
Figure 1 Time Course of Viral Myocarditis

Time course of viral myocarditis in 3 phases (derived from murine models). The acute phase of myocarditis takes only a few days, whereas the subacute and chronic phase covers a few weeks to several months. Modified from Kawai (22).

High Prevalence of Viral Genomes and Multiple Viral Infections in the Myocardium of Adults With "Idiopathic" Left Ventricular Dysfunction

Uwe Kühl, PhD, MD; Matthias Pauschinger, MD; Michel Noutsias, MD; Bettina Seeberg, MD; Thomas Bock, PhD; Dirk Lassner, PhD; Wolfgang Poller, MD; Reinhard Kandolf, PhD, MD; Heinz-Peter Schultheiss, MD

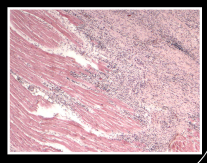
(*Circulation*. 2005;111:887-893.)



Cardiotrophic viruses
RNA 3 – 53%
DNA 3 – 23 %

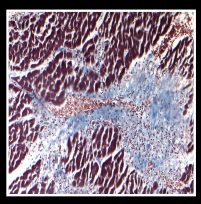
ACUTE/SUBACUTE MYOCARTIDIS

- ✓ RHYTHM DISTURBANCES
  - ✓ Atrioventricular and intraventricular block
  - ✓ Ventricular arrhythmias
    - ✓ Frequent Ventricular ectopic beats
    - ✓ Monomorphic ventricular tachycardia
  - ✓ Supraventricular arrhythmias
    - ✓ Atrial fibrillation
    - ✓ Focal atrial tachycardia
    - ✓ Atypical atrial flutter
- ✓ DCM PHENOTYPE (ACUTE ONSET)
- ✓ SUDDEN DEATH



CHRONIC MYOCARDITIDS

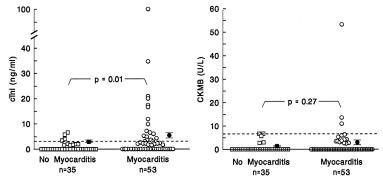
- ✓ RHYTHM DISTURBANCES
  - ✓ Atrioventricular and intraventricular block
  - ✓ Ventricular arrhythmias
  - ✓ Supraventricular arrhythmias
- ✓ DCM PHENOTYPE
  - ✓ Non familiar
  - ✓ Post- PM implantation



Elevations of Cardiac Troponin I Associated With Myocarditis

Experimental and Clinical Correlates

Stacy C. Smith, MD; Jack H. Ladenson, PhD; Jay W. Mason, MD; Allan S. Jaffe, MD



Sensitivity 34 % - Specificity 89 % - Positive Predictive 82%

Elevations of Cardiac Troponin I Associated With Myocarditis

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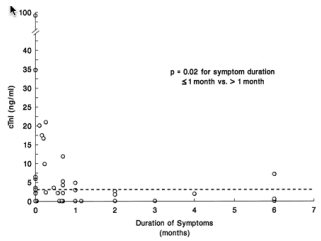


European Heart Journal (2011) 32, 897–903

CLINICAL RESEARCH
Myocardial disease

Virus serology in patients with suspected myocarditis: utility or futility?

Felix Mahfoud[1]*, Barbara Gärtner[2], Michael Kindermann[1], Christian Ukena[1], Katharina Gadomski[1], Karin Klingel[3], Reinhard Kandolf[3], Michael Böhm[1], and Ingrid Kindermann[1]

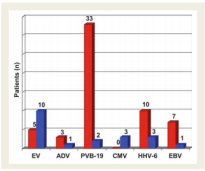


Table 2 Two by two table of virus serology and direct viral detection in EMB

	Biopsy negative	Biopsy positive	*P*
All patients, n = 124			
Serology negative	51	53	
Serology positive	15	5	0.04
IHC positive, n = 54			
Serology negative	18	30	
Serology positive	4	2	0.17
Time from initial symptoms to EMB ≤1 month, n = 76			
Serology negative	31	32	
Serology positive	9	4	0.19

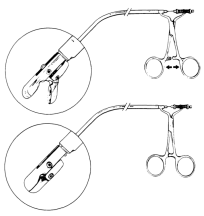
EMB, endomyocardial biopsy; IHC, immunohistology; F, Fisher's exact test.

Sensitivity 9% – Specificity 77 %

Techniques for Right and Left Ventricular Endomyocardial Biopsy

JAY W. MASON, MD
Stanford, California

May 1, 1978 The American Journal of CARDIOLOGY Volume 41



Diagnostic Yield – 70-89 %

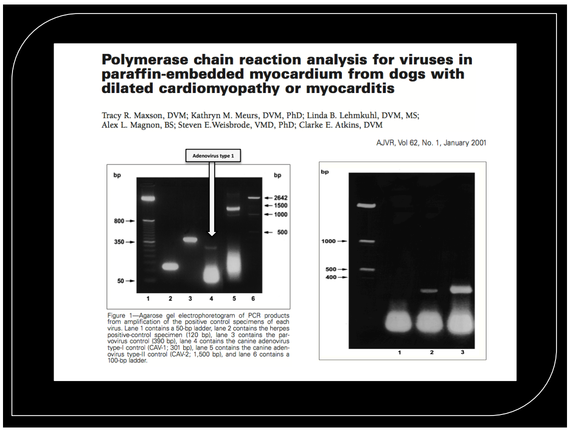
Table 1 Risks associated with endomyocardial biopsy in 546 procedures

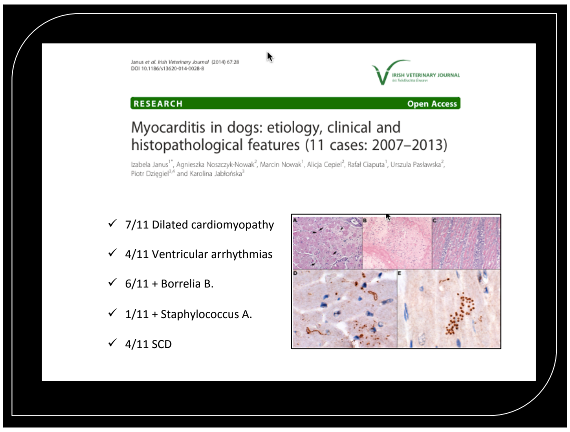
Overall 33 complications (6%)
Sheath insertion 15 (2.7%)
12 (2.0%) arterial puncture during local anesthesia
2 (0.4%) vasovagal reaction
1 (0.2%) prolonged venous oozing after sheath removal
Biopsy procedure 18 (3.3%)
6 (1.1%) arrhythmia
5 (1.0%) conduction abnormalities
4 (0.7%) possible perforation (pain)
3 (0.5%) definite perforation (pericardial fluid)
2 of 3 patients with definite perforation died

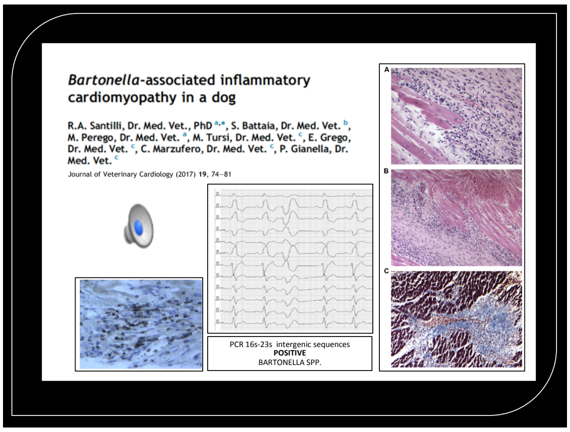
Data derived from Deckers et al.[20]

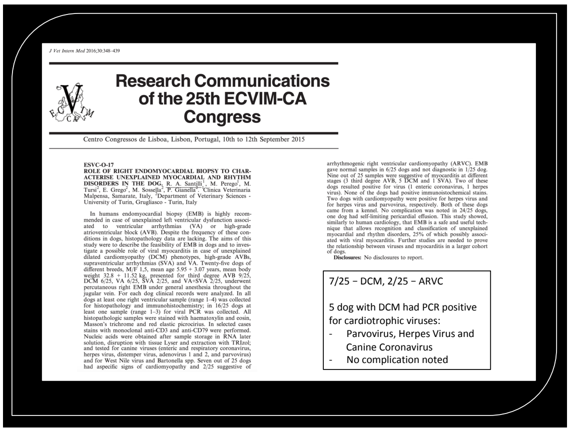
Myocarditis in Dogs

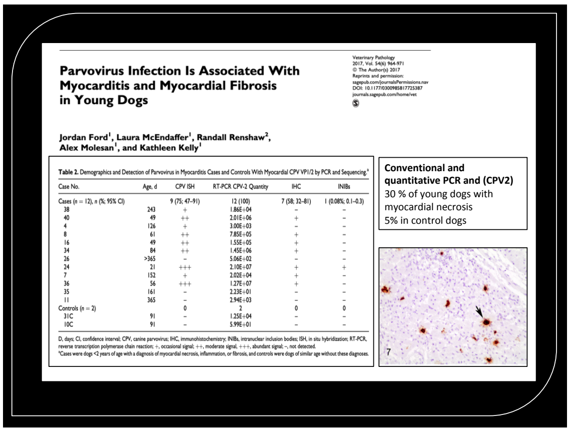
RNA VIRUS	DNA VIRUS
Distemper (1981) West Nile (2003,2006)	Adenovirus 1 (2001) CPV-2 (1986,1999)
BACTERIA	**PROTOZOA**
Bartonella spp (1999, others) Citrobacter K. (2002) Borrellia B. (1988, others)	Trypanosoma C (1994,others) Neospora C (1993, others) Leishmania I (2005) Babesia C. (2005)

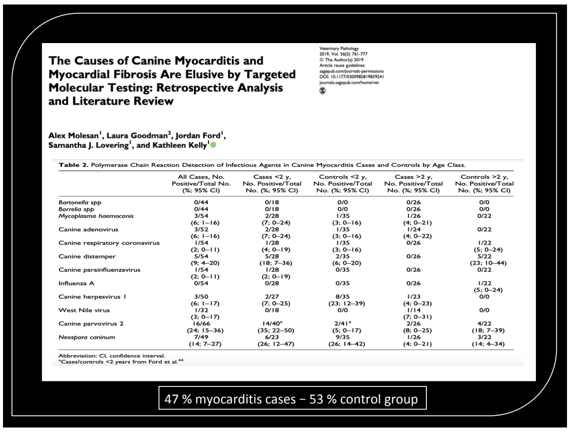

Polymerase chain reaction analysis for viruses in paraffin-embedded myocardium from dogs with dilated cardiomyopathy or myocarditis
Tracy R. Maxson, DVM; Kathryn M. Meurs, DVM, PhD; Linda B. Lehmkuhl, DVM, MS; Alex L. Magnon, BS; Steven E. Weisbrode, VMD, PhD; Clarke E. Atkins, DVM
AJVR, Vol 62, No. 1, January 2001
Adenovirus type 1


IRISH VETERINARY JOURNAL
RESEARCH
Open Access
Myocarditis in dogs: etiology, clinical and histopathological features (11 cases: 2007–2013)
✓ 7/11 Dilated cardiomyopathy
✓ 4/11 Ventricular arrhythmias
✓ 6/11 + Borrelia B.
✓ 1/11 + Staphylococcus A.
✓ 4/11 SCD


Bartonella-associated inflammatory cardiomyopathy in a dog
R.A. Santilli, Dr. Med. Vet., PhD, S. Battaia, Dr. Med. Vet., M. Perego, Dr. Med. Vet., M. Tursi, Dr. Med. Vet., E. Grego, Dr. Med. Vet., C. Marzufero, Dr. Med. Vet., P. Gianella, Dr. Med. Vet.
Journal of Veterinary Cardiology (2017) 19, 74–81
PCR 16s-23s intergenic sequences
POSITIVE
BARTONELLA SPP.


Research Communications of the 25th ECVIM-CA Congress
Centro Congressos de Lisboa, Lisbon, Portugal, 10th to 12th September 2015
ROLE OF RIGHT ENDOMYOCARDIAL BIOPSY TO CHARACTERISE UNEXPLAINED MYOCARDIAL AND RHYTHM DISORDERS IN THE DOG
7/25 – DCM, 2/25 – ARVC
5 dog with DCM had PCR positive for cardiotrophic viruses:
- Parvovirus, Herpes Virus and Canine Coronavirus
- No complication noted


Parvovirus Infection Is Associated With Myocarditis and Myocardial Fibrosis in Young Dogs
Jordan Ford, Laura McEndaffer, Randall Renshaw, Alex Molesan, and Kathleen Kelly
Veterinary Pathology 2017, Vol. 54(6) 964-971
Conventional and quantitative PCR and (CPV2)
30 % of young dogs with myocardial necrosis
5% in control dogs


The Causes of Canine Myocarditis and Myocardial Fibrosis Are Elusive by Targeted Molecular Testing: Retrospective Analysis and Literature Review
Alex Molesan, Laura Goodman, Jordan Ford, Samantha J. Lovering, and Kathleen Kelly
Veterinary Pathology 2019, Vol. 56(5) 761-777
Table 2. Polymerase Chain Reaction Detection of Infectious Agents in Canine Myocarditis Cases and Controls by Age Class.
47 % myocarditis cases – 53 % control group

Prevalence of selected cardiotropic pathogens in the myocardium of adult dogs with unexplained myocardial and rhythm disorders or with congenital heart disease

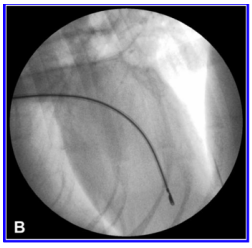
Roberto A. Santilli DMV, PhD
Elena Grego DMV, PhD
Stefano Battaia DMV, MS
Paola Gianella DMV, PhD
Massimiliano Tursi DMV
Nicola Di Girolamo DMV, PhD
Ilaria Biasato DMV, PhD
Manuela Perego DMV

(*J Am Vet Med Assoc* 2019;255:1150–1160)

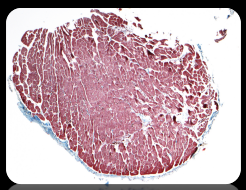
37 dog with **U**nexplained **M**yocardial and **R**hythm **D**isorders (UMRD)

- 25/37 – **Dilated cardiomyopathy**
- 6/37 – Atrioventricular block
- 4/37 – nonfamilial ventricular arrhythmias
- 2/37 – supraventricular arrhythmias

10 control dogs with **C**ongenital **H**eart **D**iseaes (CHD)



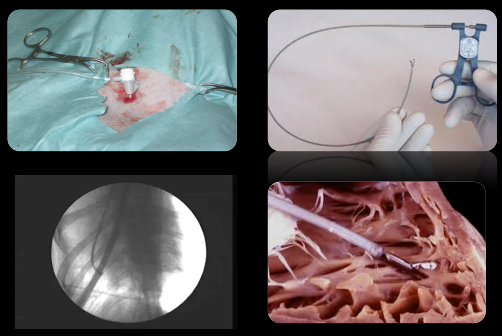
AIM OF THE STUDY



- ✓ To describe the feasibility of EMB in dogs
- ✓ To investigate a possible role of myocarditis in case of:
  - ✓ Non familial dilated cardiomyopathy (DCM) phenotypes,
  - ✓ New onset
    - ✓ High-grade AVBs,
    - ✓ Supraventricular arrhythmias
    - ✓ Ventricular arrhythmias.

MATHERIAL AND METHODS

- ✓ 47 dogs of different breeds,
- ✓ Gender
  - ✓ 30 males (5 in CHD group) and 17 females (5 in CHD group)
- ✓ Median age
  - ✓ 4 years (range 0.5 -11 years) – UMRD group
  - ✓ 2.5 years (range 0.7 – 6 years) – CHD group
- ✓ Median body weight
  - ✓ 32 Kg (range 11.7 - 64.0 Kg) – UMRD group
  - ✓ 29.7 Kg (range 15.0 – 51.0 Kg) – CHD group



Right Ventricular Endomyocardial Biopsy
3 samples RV Apex - **3** samples RV Septum

Histopathology

Light microscopy

- ✓ Formalin-fixed EMB samples were processed by routine methods, embedded in paraffin wax, and sectioned at a thickness of 5 µm.
- ✓ Twenty-four serial sections were prepared for each EMB sample;
  - ✓ 6 were evaluated histologically stained with H&E stain for morphological evaluation (slides 1, 6, 11, 16, 21, and 24)
  - ✓ 6 were evaluated histologically stained with Masson trichrome stain to detect collagen deposition. (slides 2, 7, 12, 17, 22, and 23)
- ✓ Each EMB sample was assessed by a single individual (MT) for evidence of myocyte hypertrophy, sarcoplasm vacuolization, fibrosis, and lymphocytic inflammation.

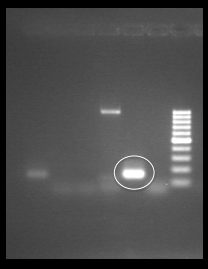
Immunohistochemical analysis

- ✓ 12 were used for immunohistochemical analysis.by use of the standard avidin-biotin-peroxidase complex method.
- ✓ Sections were then washed with PBS solution, incubated for 60 minutes with the primary antibody
  - ✓ polyclonal rabbit anti-human CD3 antibody [1:50 di-lution] to detect T lymphocytes T
  - ✓ monoclonal mouse anti-human CD79 anti-body [clone JCB117; 1:25 dilution] to detect B lymphocytes

Morphometric analysis

- ✓ Sections stained with Masson trichrome stain were evaluated to quantify the extent of fibro-sis (calculated as a percentage of the surface area), Magnification of 2,500X
- ✓ Sections stained with H&E stain were evaluated to quantify the extent of lymphocytic inflammation (calculated as the number of lymphocytes per mm2).
- ✓ Commercial photo-editing softwarec was used.

Amplified Genomic Sequence for PCR



+ CPV-2

- ✓ To preserve nucleic acids myocardial and blood stored at - 80°C in RNA later solution
- ✓ Nucleic acids extraction
  - ✓ Phenol-chloroform technique (Qiazol, qiagen) adapted to small sample size
- ✓ ATPasi used to assess integrity of RNA extracted
- ✓ GAPDH used to assess integrity of DNA extracted

Amplified Genomic Sequence for PCR

Primers used for PCR assay, reverse transcriptase PCR assay, or real-time PCR assay to detect pathogens in EMB samples collected from dogs with UMRD or CHD during RV catheterization.

Purpose	Target gene	Primer sequence (5′ to 3′)	Reference
Control extraction			
DNA	GAPDH	F: GTTCCAGTATGATTCCACCC R: TCCCTCCACGATGCCAAA	20
RNA	Na+/K+ ATPase α	F: GCTGACTTGGTCATCTGC R: AGGTAGGTTTGAGGGGATAC	21
Pathogen detection			
Canine adenovirus 1	E3	F: CGCGCTGAACATTACTACCTTGTC R: CCTAGAGCACTTCGTGTCCGCTT	41
Canine adenovirus 2	F	F: TGTCAACAAGGTTTTGTCTTTT R: TTTTCAAGGGAGGTGCGT	42
Bartonella spp	16S-23S	F: CTTCGTTTCTCTTTCTTCA R: GGATAAACCGGAAAACCTTC	43
Borrelia burgdorferi sensu lato	26SN1–23SC1	F: ACCATAGACTCTTATTACTTTGAC R: TAAGCTGACTAATACTAATTACCC	44
Canine coronavirus	M	F: TCCAGATATGTAATGTTCGG R: TCTGTTGAGTAATCACCAGCT	45
Canine herpesvirus 1	K	F: TGCCGCTTTTATATAGATG R: AAGCGTTGTAAAAGTTCGT	46
Canine parvovirus 2	VP2	F: CATTGGGCTTACCACCATTTCCAACC R: TCAGCTGGTCTCAT	47
Canine distemper virus	N	F: GATAAAGCATGTCATTATAGTCCTAA R: CTTGAGCTTTCGACCCTTC	48
West Nile virus	NS5	F: GCMATHAGGTWCATGTGG R: GTRTCCCAKCCDGCNGTRTC	49
Toxoplasma gondii	ITS	F: TGGCGCGTTCGTGCCCGAAAT R: TGCAITTYGCTGCGKYCTTC	40
Leishmania infantum	kDNA	F: AAAGCGGGCGCGGTGCTG R: TCCCATCGCAACCTCGGTT	50

F = Forward. R = Reverse.

Proposed Classification

- ✓ Based on the histopathological and PCR results the following diagnoses were made
- ✓ **Non-specific Cardiomyopathy**:
  - ✓ mild hypertrophy with focal interstitial and replacement fibrosis and/or fatty degeneration with negative PCR;
- ✓ **Primary rhythm disturbances**:
  - ✓ presence of AVB, SVT or VA with no histological changes or with mild hypertrophy without interstitial or endocardial fibrosis with negative PCR;
- ✓ **Acute/subacute lymphocytic myocarditis**:
  - ✓ focal lymphoplasmacytic infiltrate (> 14 leukocytes/mm^2), with necrosis of non-ischemic origin and myocyte degeneration, without replacement fibrosis with positive or negative PCR;
- ✓ **Borderline myocarditis**:
  - ✓ focal *lymphoplasmacytic* infiltrate (> 14 leukocytes/mm^2) without evidence of necrosis and myocyte degeneration, no replacement nor endocardial fibrosis, with positive or negative PCR;
- ✓ **Chronic immune-mediated myocarditis/inflammatory cardiomyopathy**:
  - ✓ focal *lymphoplasmacytic infiltrate* (> 14 leukocytes/mm^2) sarcoplasmic vacuolation with replacement or endocardial fibrosis and negative PCR;
- ✓ **Chronic infective myocardial disease (CIMD)**:
  - ✓ mild hypertrophy with focal replacement or endocardial fibrosis and/or fatty degeneration and sarcoplasmic vacuolation with or without focal lymphoplasmacytic infiltrates (≥ 14 leukocytes/mm^2), and positive PCR.

Statistical Analysis

- ✓ Minimum sample size was calculated to be 23 dogs with UMRD and 6 dogs with CHD.

  Required sample size = (1.28√[p1(1 – p1) + p0(1 – p0)] + 1.96√[2p′(1 – p′)])2/(p1 – p0)2

- ✓ Distributions of age and body weight data were evaluated by means of a Shapiro-Wilk test, and summary statistics were calculated.
- ✓ Differences between dogs with UMRD and dogs with CHD regarding sex, age, and body weight were assessed with a Fisher ex-act ex-act test (for categorical variables) or Student *t* test or Mann-Whitney *U* test (for normally and nonnormally distributed continuous variables, respectively).
- ✓ Logistic regression was performed to identify fac-tors associated with the outcome (ie, detection of nucleic acid from ≥ 1 cardiotropic pathogen by PCR assay).
- ✓ Univariate logistic regression models were used to evaluate candidate variables (ie, age, sex, body weight, and type of cardiac disorder [UMRD vs CHD]) for potential inclusion in the multivariate model..
- ✓ Variables with a value of $P < 0.1$ were included in the multivariate regression model.
- ✓ Odds ratios and 95% CIs were reported to describe the strength of the association between the predictor and outcome variables. Nagelkerke *R2* values were calculated to de-termine the proportion of variability in the outcome explained by the model. No subgroup analyses were performed because of the limited sample size.
- ✓ All analyses were performed with commercially available software. Two-sided values of $P < 0.05$ were considered significant.

RESULTS

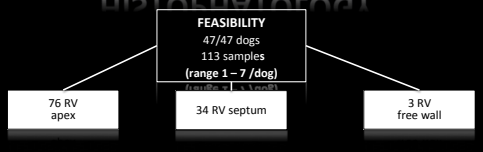
Table 1—Results of univariate logistic regression for dogs with UMRD (n = 37) or CHD (10) for which EMB samples were examined by use of PCR assays to detect nucleic acid from 12 cardiotropic pathogens.

Variable	Dogs with positive results (n = 22)	Dogs with negative results (n = 25)	OR (95% CI)	*P* value
Disease type				
UMRD	21 (57)	16 (43)	11.8 (1.3–103.0)	0.02
CHD	1 (10)	9 (90)	Referent	NA
Age (y)	5.4 (3.4)	3.7 (2.6)	1.2 (0.9–1.5)	0.07
Body weight (kg)	36.6 (15.5)	31.5 (10.7)	1.0 (0.9–1.1)	0.19
Sex				
Male	15 (50)	15 (50)	1.4 (0.4–4.7)	0.56
Female	7 (41)	10 (59)	Referent	NA

Values are reported as mean (SD) for continuous data and as number of dogs (%) for categorical data. The OR represents the likelihood of a positive result (ie, detection of nucleic acid from ≥ 1 cardiotropic pathogen) in dogs with UMRD versus CHD or in male dogs versus female dogs or the increase in the likelihood of a positive result for each 1-year increase in age or 1-kg increase in body weight.
NA = Not applicable.

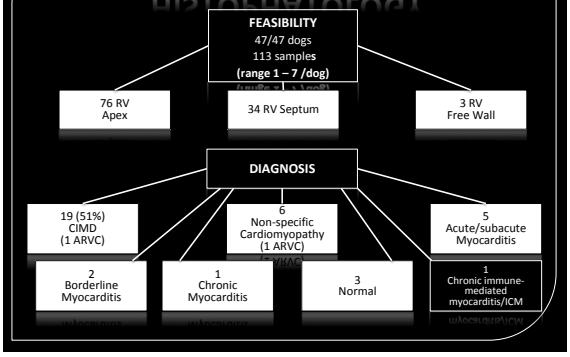
Blood samples were available from 18 of the 21 dogs with positive PCR assay results for EMB samples; all 18 blood samples were negative

HISTOPHATOLOGY



- ✓ 3/47 dogs had complications associated with the EMB
  - ✓ 1 dog perforation of the RV wall and self-limiting intrapericardial hemorrhage without cardiac tamponade
  - ✓ 2 dogs self-limiting polymorphic ventricular tachycardia during the procedure.

HISTOPHATOLOGY



GENOMIC PCR

FEASIBILITY
47/47dogs
84 samples
(range 1-3 /dog)

- 14 Positive RNA Viruses
- 4 Positive Bacteria
- 1 Positive Protozoa
- 8 Positive DNA Viruses

DIAGNOSIS
15 positive single pathogen
6 positive > 1 pathogen

16 Negative	9 Canine Distemper	6 CPV-2	5 Canine Coronavirus	4 Adenovirus 1,2	5 Bartonella	2 Herpes Virus 1	1 Leishmania
13/25 DCM	*	*			*	*	

GENOMIC PCR

FEASIBILITY
47/47dogs
84 samples
(range 1-3 /dog)

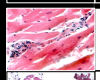
- 14 Positive RNA Viruses
- 4 Positive Bacteria
- 1 Positive Protozoa
- 8 Positive DNA Viruses

DIAGNOSIS
15 positive single pathogen
6 positive > 1 pathogen

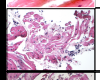
16 Negative	9 Canine Distemper	6 CPV-2	5 Canine Coronavirus	4 Adenovirus 1,2	5 Bartonella	2 Herpes Virus 1	1 Leishmania
3/6 AVB			*				

DCM Phenotype Group

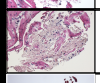
- **25 CASES**
  - 14 Tested positive for cardiotrophic pathogens
  - 5 Non-specific cardiomyopathy (1 ARVC)
  - Median CtnI – 0.57 ng/ml



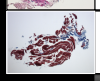
5 ACUTE MYOCARDITIS
PCR 1 + Canine Coronavirus



2 BORDERLINE MYOCARDITIS
PCR -



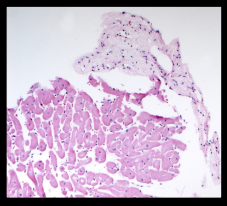
13 CHRONIC INFECTIOUS MYOCARDIAL DZ
PCR 6 + Distemper; 2 Herpes Virus 1; 1 CPV-2; 1 Adenovirus 1; 1 Adenovirus+Bartonella; 1 CPV-2+Coronavirus; 1 Coronavirus+Bartonella



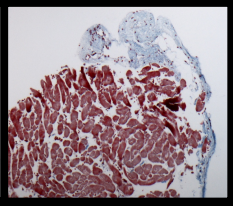
1 INFLAMMATORY CARDIOMYOPATHY
PCR -

DCM Phenotype Group

- **25 CASES**
  - 14 Tested positive for cardiotrophic pathogens
  - 5 Non-specific cardiomyopathy (1 ARVC)
  - Median CtnI – 0.57 ng/ml



EE, 10 X



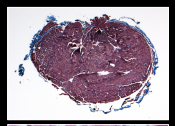
Masson, 10 X

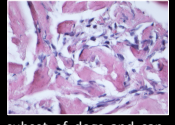
Limitations

- ✓ Small sample's size and number due to in-vivo diagnosis
  - ✓ Negative results of immunohistochemistry
  - ✓ Negative results in case of acute myocarditis
- ✓ Limited number of cardiotrophic pathogen tested
- ✓ Inability to test blood samples in all patient with myocardial PCR positive
- ✓ Impossibility to correlate if the pathogen isolated from the heart was causing the disease
- ✓ Lack of titers for tick-transmitted diseases, Neospora, toxoplasma and CTnI in all dogs.

Conclusion

- ✓ Despite evident limitation due minimal sampling of the right ventricle, endomyocardial biopsy should considered as a promising diagnostic tool in patient with
  - ✓ Non familial DCM phenotypes,
  - ✓ New onset AVB particularly in young dogs,
  - ✓ Supraventricular or ventricular arrhythmias in patients with no cardiostructural diseases.





- ✓ Viral myocarditis can be common in this subset of dogs and can induce different forms of hypokinetic heart. In such a cases EMB is crucial to guide early treatment and prevent end-stage disease.

Clinical presentation, cardiovascular findings, etiology, and outcome of myocarditis in dogs: 64 cases with presumptive antemortem diagnosis (26 confirmed postmortem) and 137 cases with postmortem diagnosis only (2004–2017)

S. Lakhdhir, DVM[a], A. Viall, DVM, MS[b], E. Alloway, DVM[c], B. Keene, DVM, MS[b], K. Baumgartner, DVM[a], J. Ward, DVM[a,*]

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Table 3 Proposed major and minor criteria for the antemortem diagnosis of myocarditis in dogs.

I. Major criteria
- A. Cardiac troponin I > 1.0 ng/mL
- B. Positive culture of blood or other bodily fluid for typical bacteria OR positive infectious disease testing (PCR, antibody serology, antigen serology, virus isolation, or microscopic visualization) for other organisms reported to cause myocarditis in dogs:
  - a) Viral: parvovirus, distemper, herpesvirus, West Nile virus, coronavirus
  - b) Atypical bacterial: *Bartonella, Leptospira, Borrelia, Rickettsia, Ehrlichia*
  - c) Protozoal: *Trypanosoma, Toxoplasma, Neospora, Leishmania, Babesia, Hepatozoon*
  - d) Fungal/algal: *Blastomyces, Coccidioides, Cryptococcus, Aspergillus, Prototheca*

II. Minor criteria
- A. Fever >102.5° F
- B. New or worsening heart murmur
- C. Inflammatory leukogram (neutrophilia and/or left shift > 0.5 × 10^3/UL), anemia, thrombocytopenia, or hypoalbuminemia
- D. Ventricular arrhythmias
- E. Decreased left ventricular systolic function (FS < 25%, LVEF < 50%, ESVI > 30, or focal hypokinesis)
- F. Heteroechogenicity of left ventricular myocardium
- G. Pericardial effusion

I. Definitive diagnosis of myocarditis:
- A. Histopathologic confirmation of myocardial inflammation on endomyocardial biopsy specimen
- B. Two major criteria
- C. One major and three minor criteria

II. Possible diagnosis of myocarditis:
- A. One major and two minor criteria
- B. Four minor criteria

ESVI, end-systolic volume index; FS, fractional shortening; PCR, polymerase chain reaction; LVEF, left ventricular ejection fraction.

Future Directions

In vivo evaluation of pathogens in dogs with myocarditis: from endomyocardial bioptic tissue to Next Generation Sequencing.

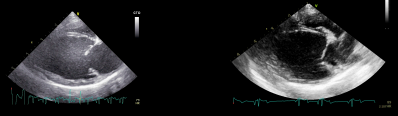
Investigators:
Roberto Santilli DMV, ECVIM-CA (Cardiology), PhD, Antonio Di Loria DMV, PhD; Paolo Ciaramella DMV, Claudia Kohl.

Future Directions

The VIPER Study
Randomized Single-blinded Study on the Efficacy of Recombinant Feline Interferon-Omega Therapy in Dogs with Viral Persistence Inflammatory Cardiomyopathy.

Investigators:
Roberto Santilli DMV, PhD, ECVIM-CA (Cardiology), Manuela PeregoDMV, ECVIM-CA (Cardiology),

Chronic Infectious Myocardial Disease (Distemper)



T0 – CHF EF 21%

T360 Post-Interferon-Omega– EF 33 %

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- ✓ Dr Manuela Perego – DECVIM-CA (Cardiology)
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- ✓ Dr Elena Grego group for genomic PCR analysis
- ✓ Referring veterinarians and owners of the dogs

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