CARDIOMYOPATHY IN BOXER DOGS

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discuss this heart condition in the boxer breed, the search for a genetic cause and call for cases and controls to help unravel its complexity

CARDIOMYOPATHY has been well recognised in boxers for nearly 30 years. In 1983, Harpster[1] described the unique clinical presentations of cardiomyopathy in this breed.

These can range from ventricular arrhythmias in asymptomatic dogs (category one), to syncopal episodes due to ventricular tachycardia (category two), to a more classical dilated form of cardiomyopathy, with poor systolic function and congestive heart failure (category three).

Subsequently, understanding of the distinct myocardial histopathological changes in this breed – characterised by fatty or fibrofatty replacement of cardiomyocytes, most pronounced in the right ventricle (**Figure 1**) – mean that this entity is now often known as arrhythmogenic right ventricular cardiomyopathy (ARVC)[2] because of its similarities to the corresponding human condition.

ARVC in boxers may have a variety of presentations. Some show classical echocardiographic findings resembling idiopathic dilated cardiomyopathy (DCM), with a rounded, hypokinetic left ventricle and left-sided or biventricular congestive heart failure (**Figure 2**).

In a large UK retrospective survey of canine DCM cases, boxers were the second most commonly affected breed (after Dobermanns)[3]. However, boxers may also show clinical signs (typically syncope) or ventricular arrhythmias (**Figure 3**) without any echocardiographic abnormalities. Such cases were omitted from this retrospective study[3], and thus, prevalence of cardiomyopathy in boxers in the UK may be underestimated.

It has been proposed that the different Harpster categories[1] represent an evolution of disease. Such cases have been recognised, but little information on longitudinal studies in boxers documenting the natural history is available. Indeed, one abstract reviewing boxer cardiomyopathy cases states that the mean age of boxers with a dilated left ventricle was younger than those presenting with arrhythmias (68.6 months versus 91.7 months)[4], which makes this evolution less likely. It is possible that these varying presentations indicate different manifestations or even different genetic aetiologies. ARVC cannot be excluded without myocardial histopathological examination.

The diagnosis of cardiomyopathy in boxers requires active exclusion of other congenital or acquired cardiac disease, or systemic causes of ventricular arrhythmias and impaired left ventricular systolic function. Echocardiography may be unremarkable in some cases with ventricular arrhythmias. Arrhythmias may be intermittent, so auscultation or a brief conventional electrocardiogram (ECG) may not identify any abnormalities.

An ambulatory 24-hour ECG recording (Holter monitoring; **Figure 4**) is more sensitive. Typically, boxers are more likely to have ventricular arrhythmias during excitement or exertion. Guidelines have been published that define a normal boxer (less than 50 ventricular premature complexes [VPCs]/24 hours) and an affected boxer (more than 100 VPCs/24 hours)[5]. However, it should be noted there can be a greater than 80 per cent day-to-day variation in the number of recorded VPCs/24 hours[6].

The complexity of the arrhythmias (couplets, triplets and runs) is also useful, and a grading scheme has been proposed[7]. Cardiac biomarkers have been investigated as possible methods of screening boxers; cardiac troponin-I may identify affected boxers, and levels are correlated with the number of VPCs/24 hours[8], but brain natriuretic peptide (BNP) is not useful at distinguishing between normal and affected boxers[9].

Boxer cardiomyopathy treatment depends on the presentation. Anti-arrhythmic medication is indicated in boxers that are symptomatic or have malignant ventricular arrhythmias on Holter monitoring. Sotalol or mexiletine, combined with atenolol, have been shown to be an effective regimen[7]. Boxers with congestive heart failure and myocardial dysfunction should be treated with conventional therapy (including furosemide, an ACE inhibitor, pimobendan and spironolactone).

Elucidating the genetic cause of boxer ARVC

The familial nature of boxer cardiomyopathy has been well recognised, both in the UK[10] and in North America[11,12].

Inheritance is most consistent with an autosomal dominant transmission[12]. Subsequently, a number of genes implicated in human familial ARVC have been identified (reviewed[13,14]), indicating that it is commonly a disease of the desmosome. As well as perturbing structural cell-to-cell connection, desmosomal mutations can result in altered cell signalling and gene expression[15]. Mutations in the coding sequences of genes for desmosomal proteins have been excluded in boxers with ARVC[16]. As the known human ARVC genes had been excluded as being associated with boxer ARVC, a genome-wide association study was carried out by the same research group, led by Kate Meurs[17]. In this study, affected dogs were defined as boxers with more than 500 VPCs/24 hours on Holter, but without echocardiographic abnormalities resembling DCM or other structural heart disease.

Control boxers had fewer than 100 VPCs/24 hours, a normal physical examination and were a minimum age of six years. The genome-wide association study identified two significant loci on canine chromosome 17, and smaller peaks on chromosomes 11 and 26.

The researchers investigated the chromosome 17 loci further – one did not have any genes

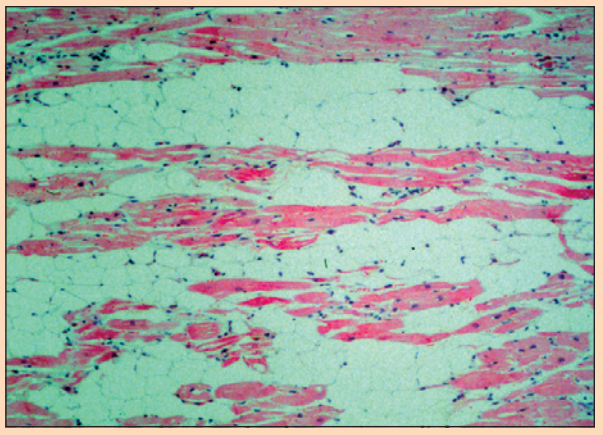


Figure 1. Histopathology from the right ventricle of a boxer dog with ARVC. Note the extensive fat infiltration separating the cardiomyocytes. This can form a substrate suitable for re-entry ventricular arrhythmias.

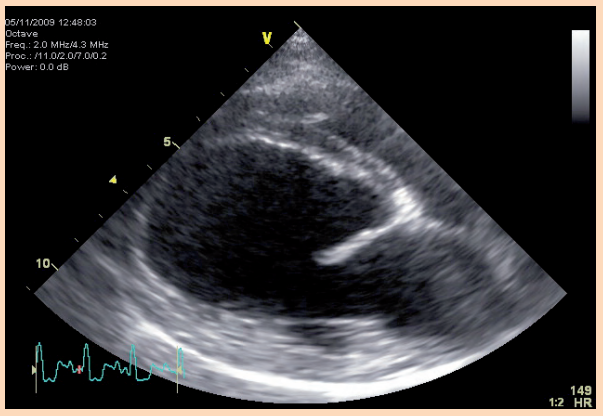
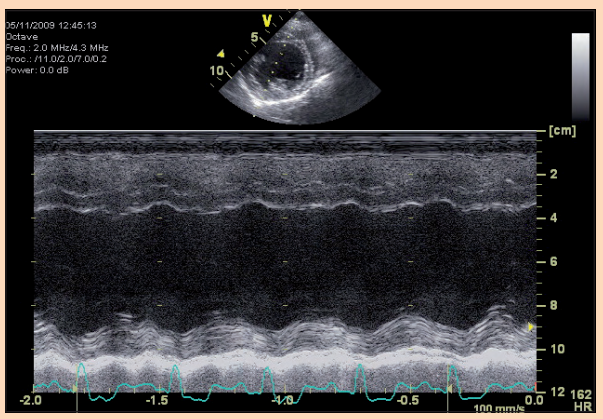


Figure 2. Echocardiogram from a boxer with a dilated, rounded, hypokinetic left ventricle, meeting criteria for the diagnosis of dilated cardiomyopathy. Figure 2a (above): 2D image, right parasternal four-chamber view. Figure 2b (below): M-mode of the left ventricle, confirming the poor contractility.



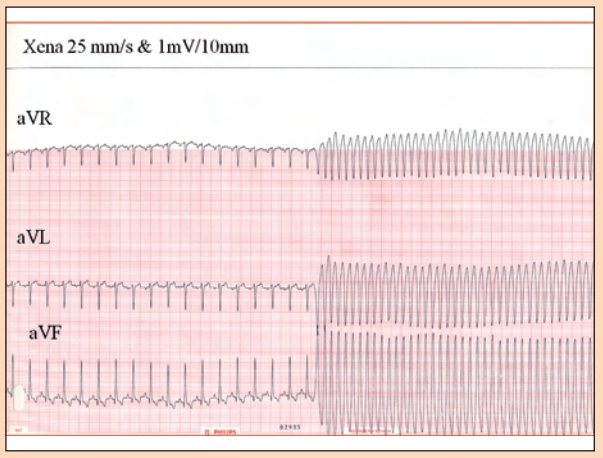


Figure 3. The typical ventricular arrhythmia in a boxer with a history of syncopal episodes. The ventricular premature complex has a left bundle block branch morphology (positive QRS in lead II and aVF), consistent with right ventricular origin. This dog was initially in sinus rhythm, but suddenly switched to ventricular tachycardia at a rate exceeding 350bpm. Sustained ventricular tachycardia may result in syncopal episodes.

that have been defined so far, although there were some flanking genes known to be associated with cardiac function, which were excluded. In the second peak, two genes were present. One of these was striatin. Mutations in the coding exons of this gene were not identified, but an eight-base-pair deletion was confirmed in the three-prime untranslated region (UTR).

The authors note that this region is likely to play a role in translation, localisation and stability of messenger RNA (mRNA), and it is rich in regulatory elements. The striatin mutation was identified in 57 of 61 ARVC boxers (either as homozygotes or heterozygotes), but it was also present in nine of 38 unaffected boxers (all heterozygotes). Boxers that were homozygous had significantly more arrhythmias than heterozygous or wildtype boxers[17]. The striatin genetic test for boxer ARVC is now commercially available in the USA (www.vetmed.wsu.edu/deptsVCGL/Boxer).

From these results[17], it can be seen that some boxers with ARVC do not have the mutation. The authors discussed possible reasons for this apparent anomaly. It could be due to inaccurate phenotyping (for example, systemic causes of ventricular arrhythmias are well recognised). It could also indicate that more than one genetic cause of boxer ARVC is apparent. Similarly, some phenotypically normal boxers did have the mutation, which could be attributed to reduced penetrance (also recognised in human ARVC).

Is striatin implicated in boxer cardiomyopathy in the UK?

To aid the authors, Kate Meurs kindly agreed to screen for the striatin mutation in UK boxers. DNA, extracted from blood stored in ethylenediamine tetra-acetic acid and surplus to that required for routine clinical pathology, was submitted from UK boxers (n=93). Of these, genotype and phenotype status was known for 84 dogs, which were further analysed.

There were 60 cases and 24 controls. Control boxers were from two sources. Some (n=14) were asymptomatic, more than eight years old and with normal echocardiographic and Holter examinations (fewer than 10 VPCs/24 hours). A smaller genetic control group (n=10) was from UK lines of boxers, which one of the authors (Bruce Cattanach) had deduced to be free from this condition, having collated data over many years. The cases included boxers with just ventricular arrhythmias, identified on ECG or Holter monitoring (ARVC) and dogs with echocardiographic changes resembling DCM.

However, all members of this latter group also had ECG or Holter evidence of ventricular arrhythmias, with apparent right ventricular origin. Therefore, both groups were combined (ARVC/DCM or cardiomyopathy). Most of these cases were related and originated from three lines of UK boxers known to have this disease in many generations.

The genotype results from 84 boxer samples submitted from the UK are shown **Table 1**. The striatin mutation is extremely common in this population of UK boxers. Both homozygous and heterozygous-positive dogs can be considered at risk with an autosomal dominant trait. The striatin mutation was identified in a homozygous or heterozygous state in 60/84 DNA samples – 71.4 per cent of all the boxers.

Further scrutiny of the results shows that no significant difference in proportions of genotypes between the cardiomyopathic boxers and the healthy boxers are apparent (**Figure 5**; Chi squared p=0.237, not significant), or between the two cardiomyopathy sub-groups or the two control sub-groups. Additionally, there was no statistically significant difference between the genotype positive (homozygotes and heterozygotes) and genotype negative dogs in the two groups, cardiomyopathy and healthy (Chi squared, p=0.849; **Figure 6**). In contrast to the USA boxers[17], the genotype was not correlated with VPC number/24 hours in this UK population, for which Holter data were available.

However, in the UK cardiomyopathy-affected boxers, the striatin genotype appeared to influence the age of diagnosis of the disease, with homozygous-positive individuals tending to be younger. However, this did not reach statistical significance (p=0.065; **Figure 7**). This possible association had not been reported in the USA study[17].

Conclusions

From the UK results, it can be seen that cases of boxer cardiomyopathy occur in dogs without having the striatin genetic mutation. Conversely, some genotype-positive dogs lead a normal life without ever manifesting the disease.

Both the UK and USA data indicate that the search for genes implicated in boxer cardiomyopathy should continue. The striatin mutation is extremely common in UK boxers, but it does not appear to segregate with disease status.

However, it may play a role as a modifier gene in this population. We did not find any association with VPC number and genotype, which is discordant with the USA results, but we have identified that the striatin genotype may influence age of disease onset.

Advice

In the UK, the striatin genotype does not appear to segregate with boxer cardiomyopathy, nor predict disease-free status. Therefore, we recommend that veterinary surgeons should advise breeders who are concerned about this disease to continue to screen for it by one or more of Holter monitoring, echocardiography and serum troponin-I measurement.

However, the disease may not manifest until later in life and at the end of a breeding career.

Figure 4. Boxer with a Holter monitor fitted. The owner keeps a diary so heart rate and rhythm can be matched with routine daily activity.

What next?

Although the USA data are compelling[17], the striatin mutation does not explain all cases of boxer cardiomyopathy in the UK – although it may play a modifying role.

continued overleaf

TABLE 1. Striatin mutation genotype from 84 samples from UK boxers

	Controls	ARVC/DCM
Homozygous positive	10	14
Heterozygous positive	8	28
Wildtype negative	6	18

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In humans, a number of gene defects can result in ARVC, and the genetic cause is only known in 40 to 50 per cent of human families[13], suggesting that other genes remain to be identified. We suspect that other genes are implicated in boxer ARVC, as already suggested by additional significant peaks in the USA genome-wide association study[17].

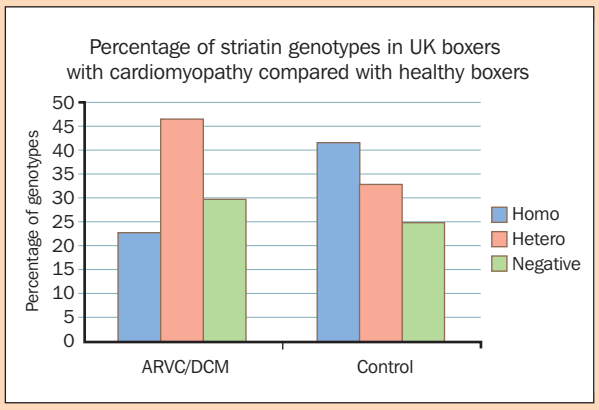

Figure 5. Results from genotyping the striatin mutation in UK boxers. There is no significant difference between clinical status of boxers (affected versus clinically healthy) and genotype (homozygous or heterozygous for the mutation, or negative for the mutation (wildtype).

The LUPA project is an EU initiative attempting to unravel canine genetic disease, with a view to elucidating human comparable conditions (www.eurolupa.org). DCM is one of these, with which two of the authors are involved (Joanna Dukes-McEwan and Hannah Stephenson). The project leaders have agreed to include boxers. A similar genome-wide association study will be run, with cohorts of unrelated boxers (at parental or, ideally, grandparental level). As boxer ARVC now appears to be influenced by more than one gene, we need cohorts of 100 individuals. This is a collaboration between the authors, the LUPA study and Kate Meurs' group with North American boxers.

Can you help?

The authors need to identify two cohorts of boxers. Whenever possible, we would like a copy of each dog's pedigree or Kennel Club registration document.

The control cohort should be equal or in advance of eight years old, without any clinical signs. Each dog should have a normal physical examination and echocardiographic examination (although mild aortic stenosis is not an exclusion), and a normal Holter examination. The cardiology service at the University of Liverpool Small Animal Teaching Hospital is willing to carry out these examinations free of charge (covered by the LUPA project). A geriatric health screen and troponin-I will be run on a blood sample, and surplus blood stored for DNA testing.

The ARVC cohort can be any age, but must have the characteristic ventricular arrhythmias documented on ECG or Holter monitor, with or without clinical signs. They may or may not have echocardiographic evidence of myocardial dysfunction or DCM, and they may or may not have congestive heart failure. Other systemic causes of ventricular arrhythmias should be excluded as far as is possible. Haematology and biochemistry are run as a health check and troponin-I is assayed, with surplus blood being stored for DNA testing.

As well as confirmed cases, we are particularly keen to identify many more control boxers to make this study possible. If you would like to discuss possible cases or controls, or if you require further information, email Dukes-McEwan at J.Dukes-McEwan@liv.ac.uk

Acknowledgements

Many thanks are due to Sonja Fonfara and Jordi Lopez Alvarez, who are both involved in screening boxers that present to the cardiology service of the University of Liverpool. Part-funding for screening of boxers was through the LUPA project, supported by the European Commission (FP7-LUPA, GA-201370).

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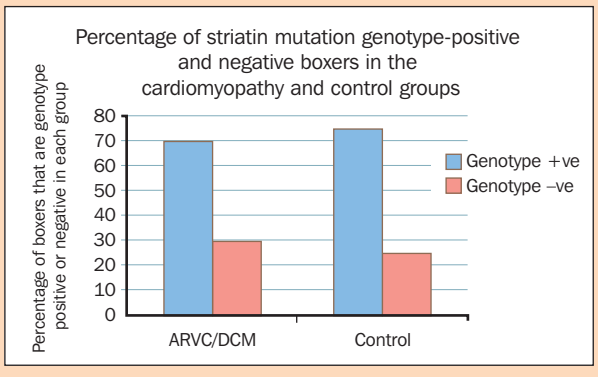


Figure 6. Results of comparing dogs that are homozygous or heterozygous positive for the striatin mutation compared with genotype negative dogs, in the cardiomyopathy and the control groups. No significant difference between proportions of genotype negative and positive dogs in the two groups exists (p=0.849).

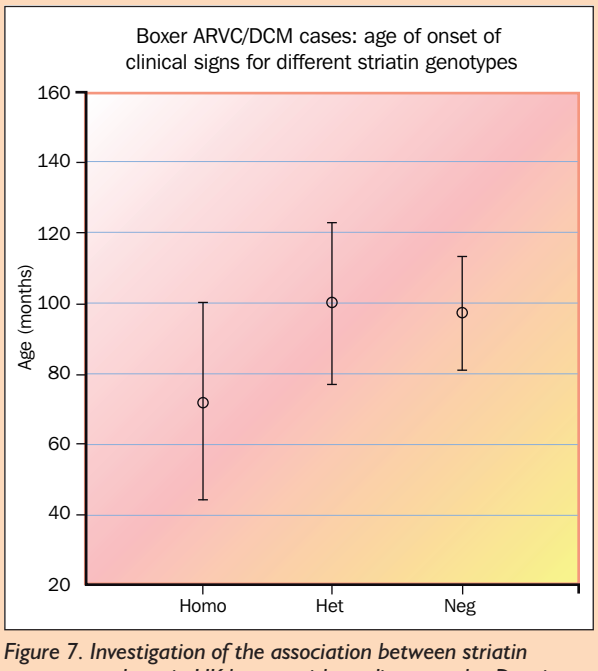
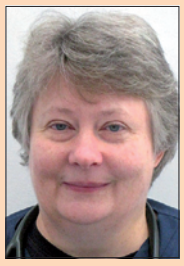


Figure 7. Investigation of the association between striatin genotype and age in UK boxers with cardiomyopathy. Despite the trend to homozygous positive dogs showing younger age of onset of clinical signs, this did not achieve statistical significance (p=0.065). The mean and range of results are shown.

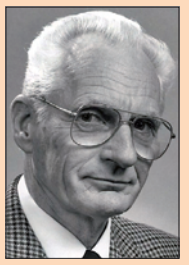
JOANNA DUKES-MCEWAN is a 1986 University of Glasgow graduate. She has worked in general mixed practice in Hereford and Canterbury, and small animal first opinion and referral practice in Glasgow. Her academic career included posts as a house physician and a residency at the University of Glasgow, and a temporary lectureship at the University of Edinburgh, where she did her PhD and a postdoctorate in studies of dilated cardiomyopathy in Newfoundland dogs. She returned to Glasgow as a senior cardiology lecturer, but is now at the University of Liverpool, where she is a senior lecturer and head of the cardiology service. She holds both the RCVS and ECVIM-CA diplomas in cardiology, and is a recognised as a specialist in cardiology in Europe and by the RCVS. She was awarded the BSAVA Blaine Award in 2008 for veterinary cardiology and teaching.



HANNAH STEPHENSON graduated from the University of Glasgow in 2005 and then spent two years in mixed practice. She did a rotating small animal internship at the RVC before being employed as a research assistant at the University of Liverpool. She has been a resident in veterinary cardiology at the University of Liverpool Small Animal Teaching Hospital since October 2009.

PAUL WOTTON graduated from the University of Bristol in 1975, where, after a spell in practice and a year as house physician, he was engaged in clinical work and research in small animal cardiology for two years, leading to a PhD. After four years as a lecturer at the RVC he returned to Bristol as a lecturer in small animal medicine. In 1993 he received the BSAVA Blaine Award and, in 1996, gained the RCVS diploma in veterinary cardiology. In 1998 he moved to the University of Glasgow's veterinary school as a senior lecturer in cardiopulmonary medicine. He worked at Davies Veterinary Specialists, Hertfordshire, from 2001 to 2007, returning to Glasgow as a senior university clinician in veterinary cardiology in August 2007. He is an RCVS-recognised specialist in veterinary cardiology.

BRUCE CATTANACH graduated with a BSc with honours in agricultural science from the University of Durham in 1955, subsequently gaining his PhD at the University of Edinburgh, pioneering chemical mutation work in mice. After two years postdoctoral work in the radiation biology laboratory in Oak Ridge, Tennessee and a brief return to Edinburgh, he moved to California where his attention shifted towards investigations into the inheritance and utilisation of mutations for understanding such phenomena as X inactivation and sex determination. Returning to the UK, he joined the Medical Research Council radiobiology laboratory, becoming head of the genetics division in 1985 when, following his contributions to the discovery of imprinting, he was elected Fellow of the Royal Society. With the creation of the Harwell MRC mammalian genetics unit, he became acting director until his retirement in 1997. Bruce has always had an interest in breeding and showing dogs. In 1980 he began helping breeders deal with a variety of inherited defects. Working with veterinary specialists, he has set up effective breeding control schemes for neurological, renal and cardiac problems in a number of breeds.



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WAG offers free biosecurity advice

FARMERS on the fringes of the Welsh badger cull region can now receive free biosecurity visits from their vets.

For the second year running the Welsh Assembly Government (WAG) is funding veterinary visits to cattle producers in the bovine tuberculosis (bTB) Intensive Action Area (IAA) in west Wales.

However, the service is also being rolled out to those on the outside of the IAA, where the delayed badger cull is due to take place.

Welsh chief veterinary officer Christianne Glossop said biosecurity was an important part of her government's approach to tackling bTB.

She said: "Last year, we had a 100 per cent take up on the offer of visits and, with the offer being extended to farmers just outside the boundary of the IAA, I would urge all cattle keepers to take up the offer of a visit.

"It costs you nothing but your time, and it will help reduce the risk of infection being introduced into your herd."

Practitioners will contact clients in the IAA to organise a biosecurity appointment. After the visit, farmers will be given a step-by-step action plan to improve their biosecurity.

Last year's advice included tips on making cattle accommodation and feed stores "badger-proof" and ensuring non-susceptible livestock were grazed on areas of badger activity.

Llandysul vet Robert Price-Jones agreed it was important for producers to consider practical steps they could take to protect their businesses. "These visits are a good opportunity for me as a vet to sit down with my client to specifically spend time to re-evaluate current practices." He added: "Good biosecurity is not a guarantee of keeping bTB from the herd, but enhancing measures to keep the disease out does improve the farmer's chances of becoming or remaining disease free."

For more details, log on to www.wales.gov.uk/bovinetb