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Assessment of Cardiovascular Disease in the Donkey: Clinical, Echocardiographic and Pathologic Observations

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Assessment of Cardiovascular Disease in the Donkey: Clinical, Echocardiographic and Pathologic Observations

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Abstract

The Donkey Sanctuary (DS) owns 3500 – 4000 donkeys, estimated to be about 35% of the UK population. Although post-mortem surveys suggest a high prevalence of cardiovascular disease in donkeys, there is sparse clinical information about cardiovascular examination findings and echocardiographic findings in health and disease. In this cross-sectional study, auscultation findings were recorded, and in a subset of donkeys, echocardiography was used to screen for structural and functional cardiac disease. 202 donkeys were examined; 117 geldings and 85 females. Heart sounds S1 and S2 were detected in all donkeys, but none had audible S3. S4 was detected in 9 (4.5%; significantly older than those without S4; $P<0.001$). A heart murmur was detected in 4 donkeys. Echocardiography identified these to be due to a ventricular septal defect in one, and aortic regurgitation in 3. An additional 43 donkeys had echocardiography. A further 10 donkeys were identified to have aortic insufficiency, but no other valvular regurgitation. 76/202 donkeys subsequently underwent necropsy. Three showed degenerative aortic valve changes. One donkey had nodular lesions in the intima of proximal aorta and sinus of Valsalva. Histopathology showed multifocal chronic nodular eosinophilic arteritis, consistent with verminous arteritis. The DS pathology database identified other similar cases.

Key words:

Equus asinus, cardiac ultrasound, verminous arteritis, ventricular septal defect, aortic regurgitation

Introduction

The donkey (*Equus asinus*) is an important working animal in many areas of the world, but in the U.K., donkeys are predominantly companion or leisure animals. The total number of donkeys worldwide has been estimated as 44 x 10^6 but in the UK, estimated numbers are 10 000 (Starkey and Starkey 1996). Approximately 35% of these UK donkeys are maintained by The Donkey Sanctuary (DS), Devon.

Given the importance of this animal for work in many parts of the world there is a dearth of information about the donkey and it is often wrongly treated as a small horse (Senior 2013). There are publications about physiological variables for donkeys for working animals (Ayo and others 2008; Maloiy and others 2003; Matthews and others 1998; Mueller and others 1994) including clinical pathology reference data (Folch and others 1997; Palanivel and others 2008; Stanisic and others 2015). Parasitism is of considerable importance in this species, and a range of publications reflect this (Asefa and others 2011; Bu and others 2009; Matthews and Burden 2013; Tolliver and others 1985; Uslu and Guclu 2007; Wells and others 1998). A large, retrospective review of post-mortem results from 1444 sedentary donkeys (DS records) has recently provided valuable information about the frequency of conditions resulting in donkey morbidity (Morrow and others 2011). The mean age was 30.6 years, so geriatric diseases are likely to be over-represented. Valvular (33%) and myocardial heart lesions (11.2%) were not infrequent (Morrow and others 2011), although severity of these were not assessed. Since this survey reported pathological evidence of valvular heart disease in one-third of donkeys, it is surprising that there is sparse clinical information regarding the pre-mortem diagnosis of cardiac disease in donkeys, or the clinical relevance of lesions identified. Heart murmurs and degenerative valvular disease are common in geriatric horses (Ireland and others 2012; Sage 2002), particularly affecting the aortic valve (Else and Holmes 1972a). There are individual case reports of pacemaker implantation in donkeys with third degree atrioventricular

block (Pibarot and others 1993) and a published letter about cardiac myopathy in a donkey foal (Dyson 1975).

Echocardiography is increasingly available as a tool to non-invasively screen for or diagnose cardiac disease in all species. Echocardiographic studies of healthy donkeys have been published in the literature to provide reference intervals (Amory and others 2004; Delvaux and others 2001; Hassan and Torad 2015), but information about structural or functional cardiac disease is lacking in this species, particularly in geriatric donkeys.

In the pathological survey (Morrow and others 2011), the prevalence of aneurysms was 43.1% (typically the cranial mediastinal artery) and prevalence of other vascular disease was 60.9%, with both occurring together in some animals (32.9%). Whether vascular disease is an ageing change in donkeys, or is associated with Strongyle infestation as in horses (Else and Holmes 1972a) is not clear.

The aims of this observational, cross-sectional study were therefore (i) to screen a selection of apparently healthy, sedentary donkeys at the DS over a wide age range and to document auscultation findings including presence and frequency of arrhythmias and heart murmurs, (ii) to use echocardiography to screen for structural or functional heart disease in those with murmurs and a subset without, (iii) to review The Donkey Sanctuary Pathology Database for evidence and prevalence of cardiac and vascular disease, including some donkeys assessed by echocardiography. Finally, we wanted to compare veterinarians' perceptions of the importance of heart disease in donkeys with the frequency of heart disease identified in this population at the DS.

Materials and Methods

Donkeys and environment

Donkeys were owned by the DS, near Sidmouth, Devon, UK. They were maintained in groups, under similar conditions and management, housed in barns, with free access to an outdoor yard and grazing. Individuals were identified by name and number on collars. Clinical records for each donkey were available, since their arrival time at the DS. Age, gender and weight were retrieved. Current diet, whether on supplemental feed, recent faecal egg count and concurrent illnesses were all noted. Donkeys were examined by one observer (SLR) between over two visits in February 2010 (4 days) and October 2012 (3 days).

Physical examination and auscultation

Individual donkeys were randomly selected based on their natural curiosity and willingness to be approached and examined by the examiner. The examinations were carried out during quiet periods, at a time when the donkeys were not being fed or otherwise handled. The weather was calm and dry, without wind.

Information recorded included demeanour, body condition score, respiratory rate and effort. Cardiac auscultation was systematically carried out (Blissitt 2010) using a Littman Cardiology III stethoscope over 5 – 10 minutes. The coat was not clipped or shaved prior to auscultation. Pulse quality was assessed from the facial artery. Heart rate and rhythm were recorded. The heart sounds detected (S1, S2, S3, S4) were noted for each case. The presence of any heart murmur was noted, and characterised by point of maximal intensity, grade of murmur (out of 6, Levine classification (Patteson and Cripps 1993) and timing. Body condition score out of 5 was determined (Svendson 2008).

2 Dimensional (2D) and colour flow Doppler echocardiography (CFDE)

2D and CFDE was performed to investigate heart murmurs detected and to assess presence of clinically silent cardiac lesions. All donkeys with murmurs were included, and 43 donkeys with normal auscultation results were randomly selected from the population. Transthoracic echocardiography was performed using an Esaote MyLab30 Vet Gold ultrasound machine with a 2.5-3.5 MHz phased array sector transducer. Right parasternal standard cardiac 2D views were obtained in standing donkeys as previously described for horses (Marr and Patteson 2010), after clipping and applying gel. An electrocardiogram was simultaneously acquired to time cardiac events. The presence of structural heart disease and cardiac remodelling was assessed by 2D echocardiography. CFDE was used to screen for the presence of any turbulent flow, indicated by the presence of colour variance. Each valve was interrogated, to identify the presence of valvular regurgitation and the inter-atrial septum and interventricular septum were assessed for shunting congenital heart defects (Marr and Patteson 2010; Marr and Reef 1995; Reef 1995). Severity of aortic regurgitation was semi-quantitatively assessed by jet area and estimating width of the aortic jet at its origin compared with aortic annulus diameter (Marr 2010b; Reef and others 2014).

Pathology

Some of the donkeys in the auscultation part of the study which died or were euthanased, underwent full post-mortem examination at the DS as previously described (Morrow and others 2011). Cause of death / euthanasia was retrieved and post-mortem reports reviewed, particularly in regard to cardiac or vascular lesions.

The full DS pathology archive of all post-mortem examinations of donkeys which died / were euthanased between 2006 and 2010 was searched for “cardiac lesions” and cardiac death, and numbers of cases retrieved.

Questionnaire

To investigate the opinions of practising veterinary surgeons working in 12 equine, large animal or mixed veterinary practices from different geographical regions on whether cardiac disease is a significant problem in pet donkeys, a survey was sent out. A total of 104 veterinary surgeons were invited to participate.

Statistical methods

Data were recorded in an Excel spreadsheet. Data were imported into SigmaPlot 13.0 for Windows (Systat software Inc.). Basic descriptive data are reported as means and standard deviation if data were normally distributed or as medians (minimum – maximum) if not. The Shapiro-Wilk test was used to assess for normality. Comparisons between males and females used the Student's unpaired two-tailed T-test for normally distributed data, or the Mann-Whitney test for non-normal distribution of data. Linear regression was used to explore associations between sets of variables.

For the questionnaire data, Chi squared analysis was used to assess the opinions of veterinarians as to the importance of clinically evident heart disease in donkeys (yes / no) with experience of examining donkeys (0, <6 and >6 donkeys/year).

A P value of <0.05 was accepted as representing statistical significance.

Results

The donkey population evaluated

A total of 202 donkeys underwent physical examination by auscultation. There were 117 males (all geldings) and 85 females. The ages, weights and body condition scores of donkeys are given in Table 1. There was no significant difference in ages between the genders, but females were significantly lighter, despite having a larger weight range than the geldings (Table 1; Figure 1); BCS was not significantly different between genders. Respiratory rate counted prior to handling in the auscultated donkeys was 20 breaths / minute, with no difference between males and females (Table 1). Respiratory rate showed a negative association with body weight and a positive association with advancing age (Table 2; Figures 2A, 2B).

Auscultation findings and echocardiographic assessment of heart murmurs

The median heart rate was 40 bpm (range 36 – 80 bpm) for the whole population, with no significant difference between the genders (Table 1). There was a weak positive association between heart rate and respiratory rate (Table 2; Figure 2C). The predominant heart rhythm evident on auscultation was regular sinus rhythm. In three donkeys, occasional second degree atrioventricular block was identified. No donkey had a pathological arrhythmia identified. All donkeys had S1 and S2 heart sounds clearly audible. S3 was not detected in any donkey. Only nine donkeys (4.5%) had an audible S4; five geldings and four females. The mean age of the donkeys with audible S4 (33.78 ± 3.90 years) was significantly older than those without (24.45 ± 8.00 years) ($P<0.001$).

A heart murmur was identified in 4 donkeys. One 10 year old gelding had a bilateral grade 2/6, basilar early to mid-systolic murmur, without any diastolic component. On investigation by echocardiography, this was confirmed to be due to a restrictive ventricular septal defect (VSD) (Figure 3A) associated with aortic prolapse (Figure 3B), but no significant aortic regurgitation. Peak VSD velocity recorded was 3.8 m/s, despite lack of optimal alignment with the VSD jet, suggesting

preserved normal left ventricular to right ventricular pressure gradient. The other three, all geldings, aged 28, 29 and 35 years old, all had left basilar diastolic decrescendo murmurs, graded 2, 3 and 5/6 respectively, confirmed on echocardiography to be due to aortic regurgitation (moderate in two, severe in the donkey with the grade 5/6 murmur) (Figure 4A; 4B). Pulse quality was considered normal in each. These three all had sinus rhythm, with heart rates of 40, 40 and 66 bpm respectively. One of these donkeys, the 35 year old with a widely radiating grade 5/6 diastolic murmur due to severe aortic regurgitation was subsequently euthanased and underwent necropsy, confirming aortic valve thickening (Figure 5A), consistent with degenerative valvular disease (Else and Holmes 1972a).

Echocardiographic findings

An additional 43 donkeys which had not had any cardiac auscultation abnormality, from the original 202 donkeys, also underwent 2D and CFDE. A total of 21.7 % of auscultation-normal donkeys were therefore examined. The simultaneously acquired ECG showed regular sinus rhythm in 46/47 donkeys, and one donkey (auscultation normal) showed occasional, physiological, 2nd degree atrioventricular block. Normal 2D and CFDE examinations were recorded in 33 (76.7%). Ten donkeys (five geldings; five females) were identified to have aortic insufficiency (AoI) (23.3%) in the absence of an audible diastolic heart murmur. AoI was mild in nine cases and one case had moderate AoI. Aortic valve abnormalities in a total of four cases were identified on 2D echo of those with AoI on CFDE, with aortic valve prolapse of the right coronary cusp in one mild case and aortic valve thickening and increased echogenicity in all four.

Of these 10 cases with CFDE AoI, eight donkeys underwent subsequent post-mortem examination. Two donkeys had thickened aortic valves coded at post-mortem examination, which had also been noted on echocardiography (including the moderate case), but two did not.

When heart murmur free AoI donkeys (N=10) were compared with heart murmur free normal echo donkeys (N=33), the AoI donkeys tended to be older (median age 29; range 25 – 44 years versus median age 23; range 3 – 45 years) but this did not achieve statistical significance (P=0.107). Including the three donkeys with diastolic murmurs associated with AoI (median age 29 years), the older age of the AoI donkeys was still not statistically significant (P=0.077). There was no difference in weight (P=0.730) or heart rate (P=0.593) between the groups.

No mitral, tricuspid or pulmonic regurgitation was identified in any of the donkeys by CFDE from these right parasternal views.

Pathology

Out of the 202 donkeys auscultated, 76 (37.6%) underwent full post-mortem examination. Five had died under treatment or were found dead, and 71 had been euthanased for various reasons (Table 3), with the major reasons for death or euthanasia identified at post-mortem being liver disease, colic or gastro-intestinal conditions and respiratory diseases. Of the post-mortem examination population, there were 39 geldings and 37 females.

Of the 76 donkeys with post-mortem data, 12 had prior echocardiography as part of this study. The echocardiography was normal in three and showed AoI in 9 out of the 12 (1 severe, 1 moderate and 7 mild cases). Post-mortem coding of “thickened aortic valve” was only reported in three of these cases (1 severe (Figure 5B), 1 moderate and 1 mild case). Aortic valve thickening was not reported in the three echocardiographically normal cases, but one 40 year old gelding (Figure 6A) from the echo-normal group had nodular lesions within the proximal aorta including the sinus of Valsalva and sinus aspect of the aortic valves themselves (Figures 6B; 6C).

Histopathology was obtained from these lesions (Figure 7A-C), which showed multifocal chronic nodular eosinophilic arteritis with chronic intimal fibrosis (arteriosclerosis) and intra-lesional mineralization consistent with chronic degenerative disease of the intima. Although parasites were

not evident in the sections, the lesions were most suggestive of chronic arteriosclerotic plaques described in other species (including horses) due to verminous arteritis (Else and Holmes 1972a). No gross myocardial lesions were identified, but systematic myocardial histopathology was not carried out.

From the full pathology archive from necropsies carried out at the DS, the total number of post-mortems and the number of donkeys identified with "cardiac lesions" as a major code are noted by year (2006 – 2010) in Table 4. These were either valvular or vascular; myocardial lesions were not coded as a major finding in these numbers. Similar nodular lesions within the proximal aorta as described above were reported.

Questionnaire

A total of 35 respondents out of 104 invitations (response rate 33.7%) replied to the questionnaire regarding opinions and familiarity with donkeys and heart disease in donkeys (data shown in Figure 8). Only 25 vets reported examining a total of 176 donkeys over the preceding year. Only one vet reported the presence of a heart murmur in a single donkey, and none of the respondents had treated cardiac disease in this species. However, of the vets treating donkeys, 10/25 vets considered that donkeys were subject to cardiac disease and 15/25 did not. The vets seeing more donkeys (average 7.9 donkeys/vet/year) thought that cardiac disease was not common, in contrast to those who did consider it a clinical problem (average 5.8 donkeys/vet/year). However, there was no statistically significant difference between vets considering heart disease was important (yes / no) and whether they examined >6, <6 or 0 donkeys a year (P=0.187).

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Discussion

This study identified a low prevalence of clinically important cardiac disease across a wide age range in this representative subset of the UK donkey population (estimated to be 5 – 6% of the total cared for by the DS). Both echocardiography and post-mortem examination identified degenerative valvular disease, particularly affecting the aortic valve, similar to horses. This tended to be in older donkeys although this did not achieve statistical significance, likely due to low numbers.

The physical examination findings, in particular heart rate and respiratory rate, are in accordance with other publications from healthy, non-working (Delvaux and others 2001; Svendsen 2008) and working (Ayo and others 2008) donkeys. Sinus arrhythmia was not identified in any donkey in our study, in contrast to 39% of donkeys (2 – 18 years old) reported by Delvaux and others (2001). Second degree atrioventricular block was documented in three donkeys, but not reported in any of the 22 in the Delvaux and colleagues' (2001) study. The association between heart rate and respiratory rate may merely reflect stress level. The association between respiratory rate and advancing age may reflect concurrent respiratory disease such as pulmonary fibrosis (Thiemann 2012).

S4 was detected in 9 donkeys, which were significantly older than donkeys without an audible S4. This is consistent with the findings in humans, where S4 is reported in a total of 15.6% healthy subjects, but increasing age increased the odds of S4 being detected (Collins and others 2005). With ageing in human patients, the left ventricle is less able to relax and ventricular filling is more dependent on atrial contraction, which may explain this finding (Lewis and Maron 1992). Detection of the transient S3 and S4 sounds is common in horses, but less common in ponies (Blissitt 2010). S3 was not detected in any donkey in this study. The presence of S3 and S4 was not commented on in other studies in donkeys (Amory and others 2004; Delvaux and others 2001; Svendsen 2008).

In this population, only 4 donkeys had murmurs identified on cardiac auscultation (2.0%). One of these was identified with a VSD. This is a fairly common congenital heart defect in foals and adult horses and ponies (Hall and others 2010; Marr 1999, 2010a; Reef 1995). The authors are unaware of published case reports about VSD in donkeys.

In three aged donkeys (1.5%), aortic insufficiency was identified by presence of a heart murmur, confirmed by Doppler echocardiography and, in one, confirmed as being associated with degenerative valvular disease on post-mortem. The prevalence is slightly less than that reported for older horses (Marr 2010b; Patteson and Cripps 1993), but the pathology in donkeys (Morrow and others 2011) is similar to that described for horses (Else and Holmes 1972a). None of the donkeys had other clinical signs consistent with haemodynamically significant aortic regurgitation (Reef and others 2014).

Heart murmurs appeared to be much less common than in horses (Patteson and Cripps 1993), but when present, in the four donkeys here, the murmur did reflect the presence of structural cardiac disease. Of particular note is that we did not identify the ejection or flow murmurs recognised frequently in thoroughbred horses (Kriz and others 2000; Patteson and Cripps 1993). This may be related to the sedentary lifestyle of donkeys at the DS compared with athletic horses. This is in contrast to the ejection-type murmurs identified in 9/22 (41%) of donkeys in another study (Amory and others 2004). Heart murmurs were not mentioned in another echocardiography study of working donkeys (Hassan and Torad 2015). In geriatric horses, 20% prevalence of heart murmurs (Ireland and others 2012), is much higher than this study. Three of the four donkeys with a heart murmur were less than the median weight of the gelding population (Table 1; weighing 142, 148 and 156 kg respectively; the heaviest weighed 184 kg). Speculatively, murmurs may be less likely to be detected in over-weight donkeys, and those with thick hair-coats.

CFDE identified AoI without an audible diastolic heart murmur, confirming its superior sensitivity over auscultation (Blissitt and Bonagura 1995). Of note, no mitral, tricuspid or pulmonic

regurgitation was identified in this study. Valvular incompetence in donkeys may be much less prevalent than in horses (Blissitt and Bonagura 1995; Kriz and others 2000; Marr 2010b). However, CFDE was only carried out from right parasternal views, and if also carried out from the left hemithorax, it is possible that more valvular incompetence would have been identified.

Only 12 donkeys underwent both echocardiography and post-mortem examination. For the three echo-normal cases, no aortic valvular pathology was noted. However, nine donkeys had AoI identified on echocardiography, but only in the severe, the moderate and in one out of the 7 mild cases classified by echocardiography, was gross aortic valve thickening documented at post-mortem. This indicates that there may be functional aortic regurgitation prior to any gross pathology, as reported in horses (Blissitt and Bonagura 1995; Marr 2010b).

The retrieved post-mortem records of the donkeys from this study were very brief, and some referred to more than one body system affected by pathology, but only the major finding was recorded in Table 3. Liver disease, gastro-intestinal disease and respiratory conditions were most frequent in this group of donkeys, and were also identified as being significant in another post-mortem survey from the DS (Morrow and others 2011). Often, euthanasia decisions were based on multiple co-morbidities affecting quality of life of each donkey. Unfortunately, we only had brief coding records of the cause of death and post-mortem findings, which likely led to some differences between this report and the study of Morrow and colleagues (2011). Vascular lesions were commonly identified in the Morrow and others (2011) study but was only coded in one case in our study. Similarly, only one case had cardiac disease listed as the most important post-mortem finding in our study (the case with severe aortic regurgitation) although aortic valve thickening was recorded in a total of four donkeys (9.2%). The severe aortic regurgitation case, and the single case where vascular disease was coded as the major post-mortem finding (the 40 year old with proximal aortic nodular lesions) were euthanized due to other quality of life issues, including laminitis, arthritis and reduced appetite rather than their cardiovascular conditions. Morrow and others (2011) reported a

prevalence of 33% with valvular disease. The differences are likely due to differing goals. In our study, we only retrieved the pathology major codes and cause of death, and did not retrieve detail of the post-mortem findings, but the goal of the Morrow and colleagues (2011) was to categorise all the conditions identified at necropsy.

The raised granulomatous lesions identified in the proximal aorta in one case in this study, and from more donkeys from the archive were thought to reflect verminous arteritis by the pathologist's report, even though no parasites were identified. Similar lesions were also described by Morrow and colleagues (2011), although their cause was not attributed to prior parasitism such as *Strongylus vasorum* infestation. There was a strong association between vascular lesions and aneurysm, particularly of the cranial mesenteric artery, speculated to be associated with ageing, possibly because endoparasites were apparently uncommon in that study (Morrow and others 2011). The location, gross pathology and histopathology findings are strikingly similar to those described in horses (Else and Holmes 1972a, b), associated with previous *Strongylus vulgaris* migration, as also shown with experimental infestation (Duncan and Pirie 1975). There is also a strong association described between Strongylosis and myocardial lesions (Cranley and McCullagh 1981). *Strongylus vulgaris* affects donkeys, including the cranial mesenteric artery (Asefa and others 2011; Tolliver and others 1985). However, to the authors' knowledge, the association of the proximal aortic nodular lesions and Strongylosis has not previously been documented in donkeys, despite evidence in horses (Else and Holmes 1972a).

The percentage of donkeys recorded in the pathology archive over each year with "cardiac lesions" as a major code was very high (approximately 70% per year). However, this includes any cardiovascular pathology, including the vascular lesions (arteriosclerosis, aortic lesions, aneurysms) as well as valvular and myocardial lesions, so these data are likely similar to those of (Morrow and others 2011). That study ranged from 2001-2008, so there will be overlap between the two studies. In the data presented here, any cardiac or vascular lesion considered to be minor would not have

been retrieved, and vascular lesions more peripheral than the proximal aorta would also not have been included, and so the numbers in table 4 likely under-represent the frequency of these findings.

The questionnaire showed that this small group of veterinary surgeons had very little experience with pet donkeys, with only 25/35 vets examining this species and the maximum number of donkeys seen per year by one vet was 35, but the respondents saw a median of 3 donkeys a year. Despite this, a minority of vets considered that clinically detectable heart disease may be a problem in the species, ranging from 30% (vets not examining donkeys) to 40% (vets examining donkeys). In fact, the vets examining more donkeys (average 7.9/vet) were less likely to consider that heart disease was significant than those examining fewer donkeys (average 5.9/vet). Only one out of a total of 176 donkeys examined was identified with a heart murmur and none were treated for cardiac disease. These results are intriguing, as they show most vets expect a much higher frequency of clinically detectable heart disease than was actually diagnosed, or that the authors identified in the DS population of donkeys. The survey of vets also confirms the dearth of veterinary literature about cardiac disease in donkeys, or lack of knowledge of sources of information. Three vets cited the Professional Handbook of the Donkey (Svendsen 2008) as their primary reference, but clinical heart disease is not mentioned in this text, although aortic valvular pathology is.

There are a number of limitations to this study. Only one observer carried out the auscultation and echocardiography, therefore inter-observer agreement or variation could not be determined. Auscultation and the detection of quiet heart murmurs can be affected by a number of factors, including environmental noise (minimised as far as possible), stethoscope quality and hearing acuity. Donkeys have thicker skin and hair coat than horses, tended to have increased fat, and also have a thicker cutaneous colli muscle, all of which may make auscultation less rewarding than in horses. It is therefore certainly possible that quieter murmurs could be missed. In addition, the donkey with a restrictive VSD, expected to have a loud right sided systolic heart murmur, only had a grade 2/6 murmur noted, which supports the “muffling” effects of the chest wall on auscultation.

Echocardiography only included the right sided views to identify CFDE evidence of valvular regurgitations, and the left sided views may have added greater sensitivity. Only a small proportion of the donkeys which underwent echocardiography also had necropsy, and we could not provide gross pathology and echocardiography images from many of the same cases, as pathology photographs were not routinely obtained. Finally, review of the pathology archive was dependent on the major coding applied at that time of necropsy, so lesions considered less significant or minor would not have been retrieved. This likely explains differences between this study and the systematic categorization of all pathological lesions by Morrow and colleagues (2011).

Conclusions

This cross-sectional study shows that donkeys may be affected by congenital and acquired degenerative valvular disease, but no donkey in this study showed significant cardiac remodelling or clinically relevant disease. Heart murmurs reflect structural heart disease. Peripheral vascular disease, with intimal nodular lesions, can affect the proximal aorta, thought to reflect prior and probably extinct Strongyle infestation.

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Tables

Table 1: Population description for the auscultated donkeys (n=202)

	All	Geldings	Females	P value between G/F
Weight (kg) Median (range)	176 (119 – 363)	180 (124 – 277)	170 (119 – 363)	**P=0.014**
BCS (/5) Median (range) (no. with data)	3.5 (1-5) (n=170)	3.5 (1-5) (n=102)	3.5 (2.5-5) (n=68)	P=0.481
Age (years) (mean ± sd) (Min-Max)	24.86 ± 8.09 (3 – 45)	24.43 ± 8.73 (3 – 45)	25.47 ± 7.12 (6 – 40)	P=0.367
Heart rate (bpm) Median (Range)	40 (36 – 80)	40 (36 – 80)	40 (36 – 80)	P=0.223
Respiratory rate (breaths per minute) Median (Range)	20 (15 – 30)	20 (15 – 30)	20 (16-30)	P=0.667

Abbreviations: BCS: body condition score, no.: number, sd: standard deviation, Min: minimum value, Max: maximum value, bpm: beats per minute.

Table 2: Associations between physiological variables in the auscultated donkey population

Association	R value	R^2	P value	Linear regression equation
Age and respiratory rate	0.393	0.154	**P< 0.001**	RR = 17.230 + (0.129 * AGE years)
Weight and respiratory rate	0.273	0.075	**P= 0.001**	RR = 21.761 - (0.00727 * WEIGHT kg)
Heart rate and respiratory rate	0.237	0.056	**P< 0.001**	RR = 16.928 + (0.0780 * HEART RATE)

Corresponding graphs are shown in Figure 2.

Table 3: Donkeys undergoing post-mortem examination (N=76)

Major post-mortem finding or cause of death	No. of cases (%)	Major post-mortem finding or cause of death	No. of cases
Cardiac	1 (1.3%)	Laminitis / feet	4 (5.3%)
Vascular	1 (1.3%)	Arthritis	7 (9.2%)
Respiratory	13 (17.1%)	Neurological	3 (3.9%)
Hepatopathy	15 (19.7%)	Collapse	1 (1.3%)
Colic / Gastro-intestinal	14 (18.4%)	Weight loss	3 (3.9%)
Dental	4 (5.3%)	Dull / anorexic	7 (9.2%)
Hyperlipidaemia	2 (2.6%)	Other	1 (1.3%) (ovarian torsion as cause of collapse)

Table 4: Numbers of donkeys identified with "cardiac lesions" as a major code by year compared with numbers of deaths and numbers of necropsies

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Year	Total died	Total necropsied	Cardiac lesions identified
2006	390	305	209 (68%)
2007	393	289	227 (78%)
2009	381	303	224 (73%)
2009	394	294	214 (72%)
2010	395	260	184 (70%)
TOTAL	1953	1451	1058 (73%)

Figure Legends

Figure 1: Weight distribution between females and geldings

Box and Whisker plots, with box defining the 25th and 75th percentile (median line included), and whiskers indicating the 10th and 90th percentiles; outlying data points shown. (P=0.014)

Figure 2. Associations with respiratory rate, by linear regression analyses

For all graphs, the regression line is shown, with 95% confidence intervals for the line, and 95% prediction intervals for the data. For R^2 and P values, see Table 2.

2A. Association between respiratory rate and body weight.

2B. Association between respiratory rate and age.

2C. Association between respiratory rate and heart rate.

Figure 3: **Ventricular Septal Defect (VSD) in a donkey gelding** (10 years old):

A. Right parasternal long axis view including left ventricular outflow tract (LVOT) during systole, optimised to show flow through the VSD by colour flow Doppler echocardiography. The * shows turbulent flow cross the interventricular septum into the right ventricle (RV).

B. Right parasternal view including aorta; early diastolic frame showing prolapse of the right coronary cusp of the aortic valve (yellow arrow). The cusp prolapses into the left ventricular outflow tract (LVOT) beyond the annulus hinge-point. Ao: proximal aorta.

Figure 4. Echo images from a 35 year old donkey gelding with severe aortic regurgitation with grade 5/6 diastolic murmur.

A. Right parasternal long axis view including the left ventricular outflow tract, with colour flow echocardiography during diastole showing the eccentric jet of aortic regurgitation. LV: left ventricle, Ao: proximal aorta, LA: left atrium. The aortic regurgitation affects the anterior mitral valve leaflet.

B. M-mode echocardiogram at mitral valve level of the same donkey with aortic regurgitation as in A; this shows diastolic flutter (yellow arrows) of open anterior leaflet of the mitral valve, between the E and A peaks, as a consequence of aortic regurgitation. IVS: interventricular septum. LVFW: left ventricular free-wall.

Figure 5. Aortic valvular lesions

A. Right parasternal long axis echocardiographic image from a 35 year old gelding, optimised to illustrate nodular thickening of the aortic valve, which was associated with aortic regurgitation. LV: left ventricle, Ao: proximal aorta. **B.** Gross pathology image from the same donkey (euthanased for quality of life reasons, associated with dental disease and chronic laminitis). One of the aortic valves cusps is thickened (yellow arrow), and was associated with moderate aortic insufficiency. A small nodular lesion can also be seen on the sinus aspect of another (non-thickened) cusp.

Figure 6. Proximal aortic intimal nodular lesions

A. Right parasternal long axis echocardiographic image from a 40 year old gelding showing no apparent major aortic valve pathology, and there was no aortic regurgitation.

B, C. Gross pathology of the sinus aspect of the aortic valves (**B**) and proximal aorta (**C**) and from the same 40 year old echo-normal gelding, showing nodular lesions. The thickened region of tip of the cusp close to the forceps (**C**) is a normal anatomical finding, the nodes of the semilunar cusps, at the point of apposition of the three cusps. The pathological nodules are deep within the sinus of Valsalva.

Figure 7. Histopathology of nodular lesions as in Figure 6B, 6C. Histopathology images courtesy of and with full permissions of Michael Rozmanec; Cytopath Ltd.

A. H and E stained, paraffin embedded section of artery. Large arrows indicate the expanded lamina intima of the vessel wall. Small arrows indicate degenerate mineralised foci. Size bar = 500 μm.

B. H and E stained, paraffin embedded section of artery. Black arrow indicates focus of vascular intima mineralisation. Red arrows indicate adjacent fibrocartilaginous / chondroid metaplasia. Size bar = 500 μm.

C. H and E stained, paraffin embedded section of artery. Black arrows indicate the junction between lamina intima and media of the blood vessel. The degenerate foci contain amorphous to granular eosinophilic coagulum – possible degenerate eosinophils or neutrophils. Size bar = 1 mm.

Figure 8. Questionnaire to Veterinary Surgeons about experience of donkeys with heart disease, and summary of responses.

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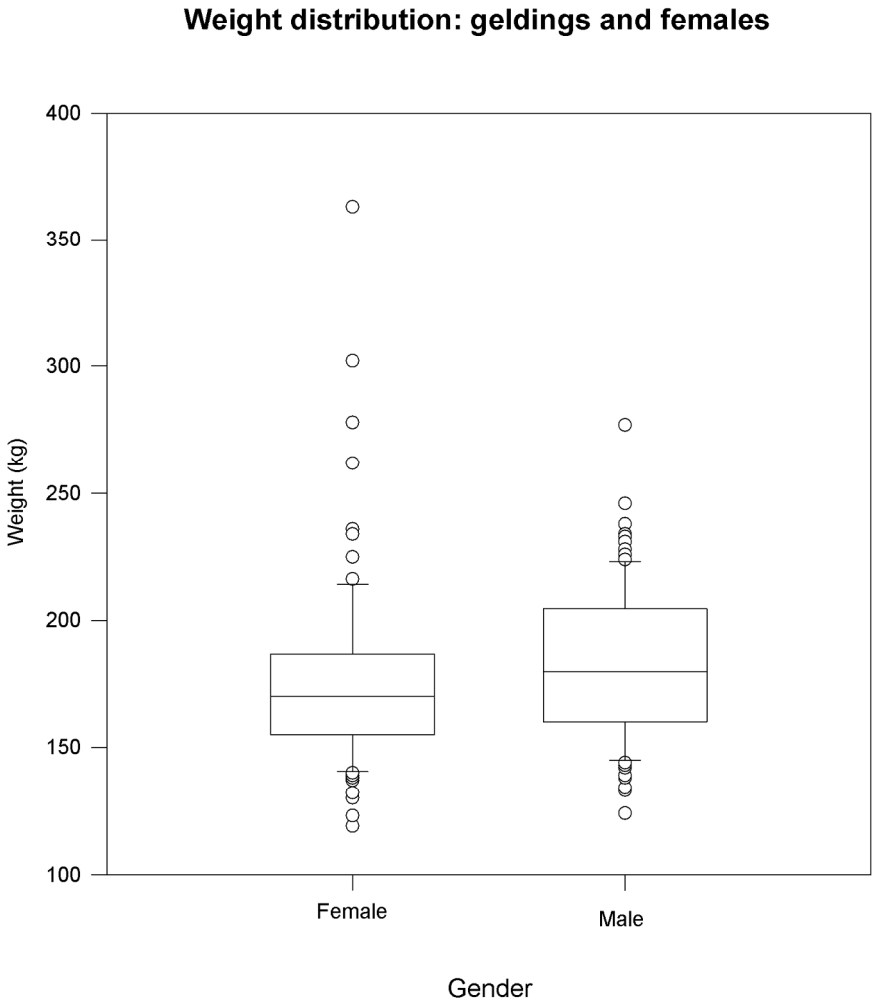

Figure 1: Weight distribution between females and geldings
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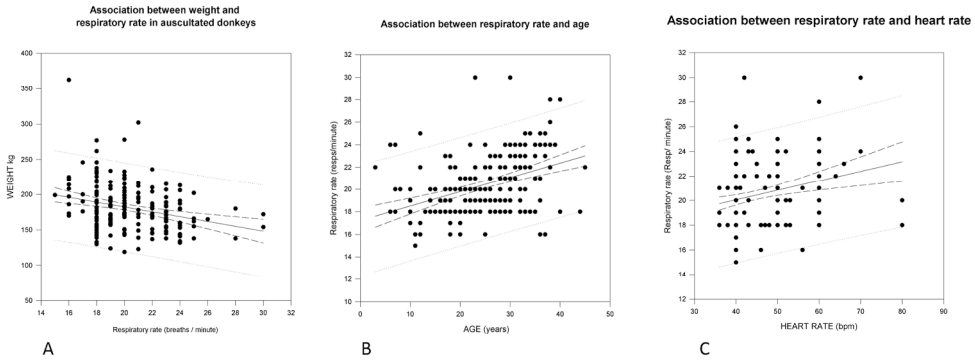

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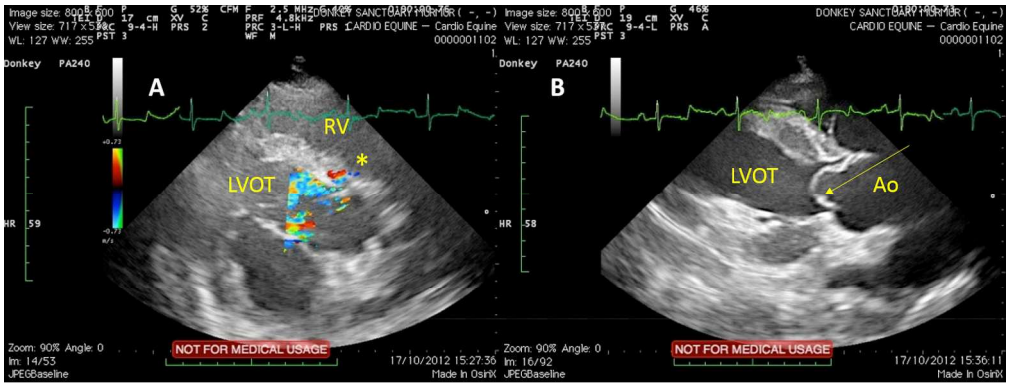

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A. Right parasternal long axis view including left ventricular outflow tract (LVOT) during systole, optimised to show flow through the VSD by colour flow Doppler echocardiography. The * shows turbulent flow cross the interventricular septum into the right ventricle (RV).
B. Right parasternal view including aorta; early diastolic frame showing prolapse of the right coronary cusp of the aortic valve (yellow arrow). The cusp prolapses into the left ventricular outflow tract (LVOT) beyond the annulus hinge-point. Ao: proximal aorta.

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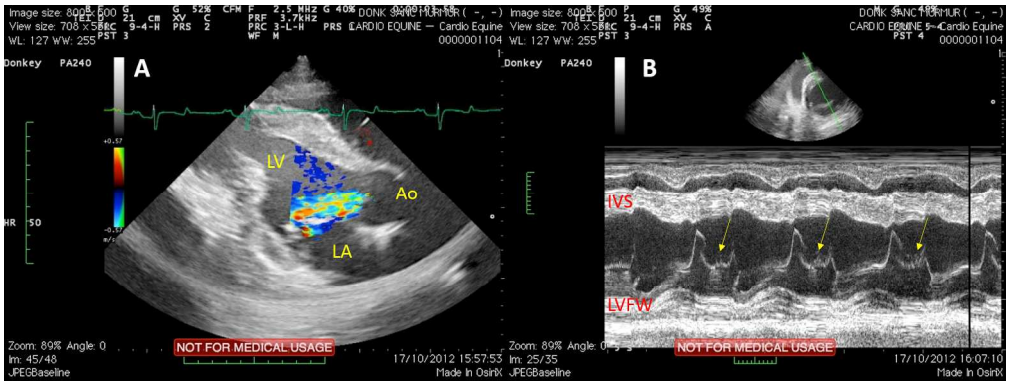

Figure 4. Echo images from a 35 year old donkey gelding with severe aortic regurgitation with grade 5/6 diastolic murmur.
A. Right parasternal long axis view including the left ventricular outflow tract, with colour flow echocardiography during diastole showing the eccentric jet of aortic regurgitation. LV: left ventricle, Ao: proximal aorta, LA: left atrium. The aortic regurgitation affects the anterior mitral valve leaflet.
B. M-mode echocardiogram at mitral valve level of the same donkey with aortic regurgitation as in A; this shows diastolic flutter (yellow arrows) of open anterior leaflet of the mitral valve, between the E and A peaks, as a consequence of aortic regurgitation. IVS: interventricular septum. LVFW: left ventricular free-wall.

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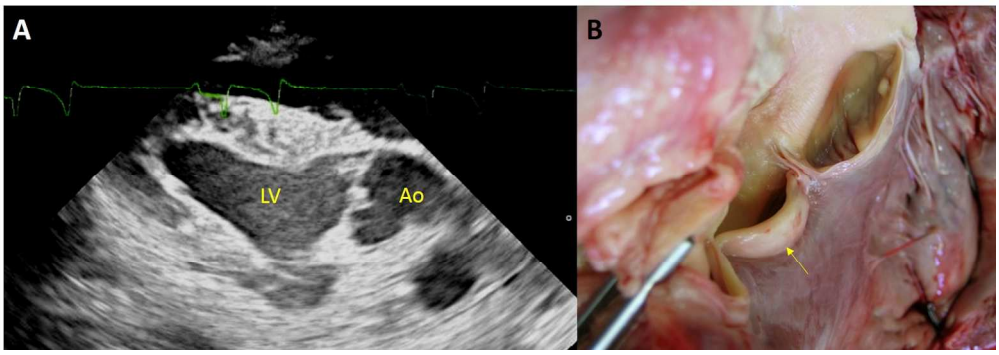

Figure 5. Aortic valvular lesions
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B. Gross pathology image from the same donkey (euthanased for quality of life reasons, associated with dental disease and chronic laminitis). One of the aortic valves cusps is thickened (yellow arrow), and was associated with moderate aortic insufficiency. A small nodular lesion can also be seen on the sinus aspect of another (non-thickened) cusp.

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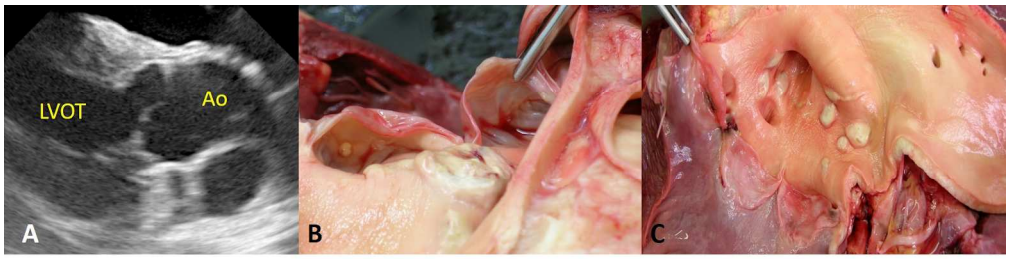

Figure 6. Proximal aortic intimal nodular lesions. ‼ ┼
A. Right parasternal long axis echocardiographic image from a 40 year old gelding showing no apparent major aortic valve pathology, and there was no aortic regurgitation. ‼ ┼
B, C. Gross pathology of the sinus aspect of the aortic valves (B) and proximal aorta (C) and from the same 40 year old echo-normal gelding, showing nodular lesions. The thickened region of tip of the cusp close to the forceps (C) is a normal anatomical finding, the nodes of the semilunar cusps, at the point of apposition of the three cusps. The pathological nodules are deep within the sinus of Valsalva. ‼ ┼

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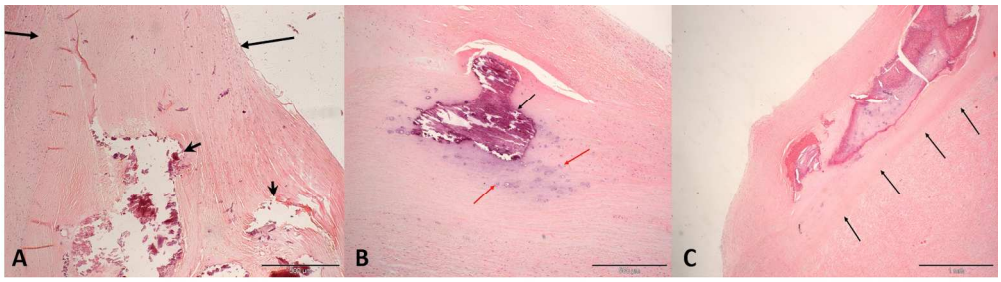

Figure 7. Histopathology of nodular lesions as in Figure 6B, 6C. Histopathology images courtesy of and with full permissions of Michael Rozmanec; Cytopath Ltd. ‼ ┼ A. H and E stained, paraffin embedded section of artery. Large arrows indicate the expanded lamina intima of the vessel wall. Small arrows indicate degenerate mineralised foci. Size bar = 500 µm. ‼ ┼ B. H and E stained, paraffin embedded section of artery. Black arrow indicates focus of vascular intima mineralisation. Red arrows indicate adjacent fibrocartilaginous / chondroid metaplasia. Size bar = 500 µm. ‼ ┼ C. H and E stained, paraffin embedded section of artery. Black arrows indicate the junction between lamina intima and media of the blood vessel. The degenerate foci contain amorphous to granular eosinophilic coagulum – possible degenerate eosinophils or neutrophils. Size bar = 1 mm. ‼ ┼

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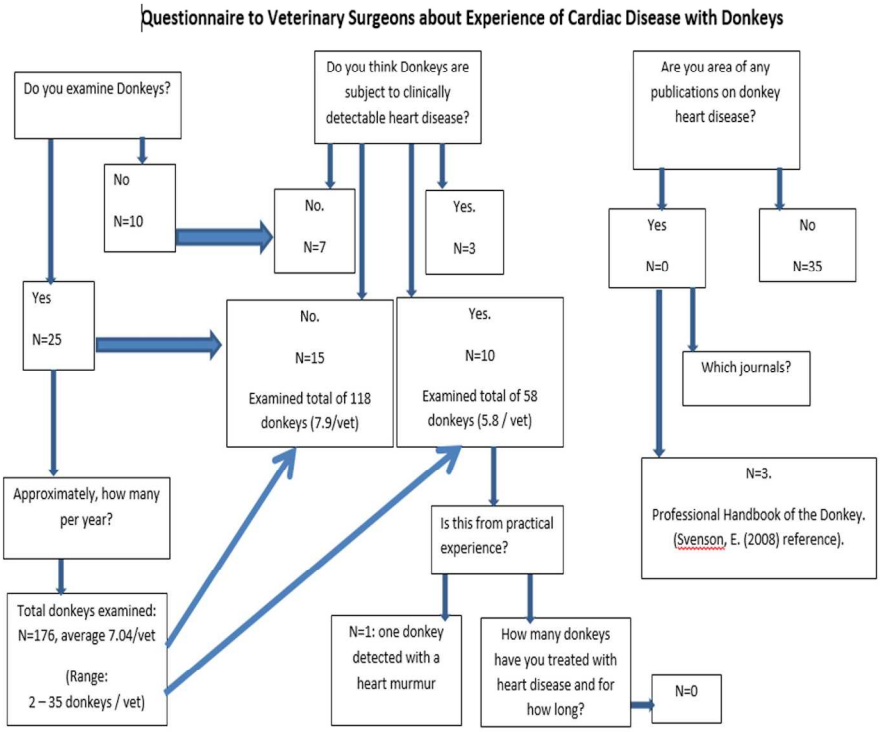

Figure 8. Questionnaire to Veterinary Surgeons about experience of donkeys with heart disease, and summary of responses.

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