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Sudden death due to aortic rupture in New Zealand sheep

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ABSTRACT

Case history: Over a period of 2 months in the spring and early summer of 2021, 13 cases of sudden death in cull ewes due to aortic rupture were diagnosed at a small number of New Zealand abattoirs.

Clinical findings: In 12/13 (92%) cases, a large blood clot was present in the thorax, and in one case the blood clot was seen in the tissues dorsal to the heart. There were no obvious signs of external trauma. The pluck (heart and lungs) or fixed aorta was submitted for histological examination in seven cases and in all of these, a tear in the aorta was found. Comparing the microscopic appearance of the proximal aorta in these seven cases to three clinically normal ewes from unaffected farms, the aortic wall thickness appeared thinner in the case ewes than the unaffected ewes. Subjectively, there was increased collagen in the tunica media in 3/7 and decreased elastin fibres in 5/7 case ewes compared to the control ewes. Further investigations on the index farm (where the first cases originated), found that the mean liver and serum Cu concentrations in 10 similarly aged, clinically normal ewes were within the normal reference range for New Zealand sheep. Similarly, the liver Cu concentrations of the seven case ewes were within the normal reference range.

Diagnosis: Aortic rupture due to an unknown aetiology.

Clinical relevance: Clinicians should be aware of this condition as a differential diagnosis for sudden death in older sheep and to assist the Ministry for Primary Industries in establishing the extent of this problem in New Zealand.

Abbreviations: EM: Electron microscopy; MPI: Ministry for Primary Industries; SoVS: School of Veterinary Science; VS: Verification Services.

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Sheep; aortic rupture; Cu; New Zealand; reduced elastin fibre

Introduction

Aortic rupture (dissecting aneurysm) is a sporadic disease in farmed animals characterised by sudden death due to internal haemorrhage through a vascular rupture, usually in the abdominal or thoracic aorta (Souto *et al*. 2017). There are reports of this condition in pigs (Shields *et al*. 1962), turkeys (Graham 1977), and bile-farmed bears (Bando *et al*. 2018) but despite the use of sheep as animal models for research into abdominal aortic aneurysm in people (Golledge 2019), reports of aortic rupture in sheep are rare. An extensive literature search completed in Web of Science and Google Scholar, on the 16 August 2022, using the search terms "aortic rupture AND sheep" and excluding articles related to human abdominal aortic aneurysm research, retrieved only three papers. Two of these papers were overseas post-mortem surveys of sheep deaths, where aortic rupture was recorded as the cause in 1/651 (0.15%) and 3/823 (0.36%) deaths (Souto *et al*. 2017; Bashir *et al*. 2020). The third paper described aortic rupture as a possible complication of diaphragmatic herniation in Texel sheep (Waine *et al*. 2019). In the study by Souto *et al*. (2017) there was a 5-cm tear where the aorta divides into the carotid artery, however, Bashir *et al*. (2020) did not record the site of the tear. A search of the School of Veterinary Science (SoVS) pathology database at Massey University (Palmerston North, NZ) yielded only two records of aortic rupture from 4,664 ovine post-mortem examinations since 1995 (H Hunt, unpublished data). The first of these was in a 6-tooth Romney cross ewe which had a 2-cm tear of the ascending aorta, approximately 6 cm from the aortic valve. The second case was in a 4-tooth Romney ewe which had a 1-cm diameter tear of the ventral aspect of the cranial part of the descending aorta, approximately 7 cm from the heart.

In pigs and poultry, an association between aortic rupture and Cu deficiency has been demonstrated (Graham 1977; Pletcher and Banting 1983). However, given the rarity of aortic rupture in sheep, no such relationship has ever been investigated in this species.

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This case study describes a Ministry for Primary Industries (MPI) investigation of ewe deaths at abattoirs prior to slaughter, where in multiple ewes, haemothorax revealed at post-mortem examination was likely associated with fatal aortic rupture. An investigation into the potential role of Cu deficiency in the pathogenesis of this condition is also described.

Case history

On 25 November 2021, an investigation by an MPI exotic pest and disease incursion investigator was launched after a Verification Services (VS) veterinarian based in Tararua reported the death prior to slaughter of four cull ewes in the lairage of an abattoir. MPI VS veterinarians verify and certify animal products from export slaughter plants to meet New Zealand legislation and overseas market access requirements. They also assess the welfare of livestock that are transported to these premises and enforce animal welfare standards. The four ewes were from two separate Gisborne sheep stations operated by the same farming trust, with ewes regularly moving between the two stations. The cause of death in each case was severe, acute, fatal haemothorax. The exact source of the haemorrhage was not located, although it was presumed to be aortic rupture, and no samples were preserved from these first four cases (outbreaks 1–2, case numbers 1–4; Table 1).

The findings were discussed with the manager of the farming trust from where these sheep originated, who stated that there had been approximately eight other sudden deaths in the same line of ewes prior to sending them to the abattoir. These eight ewe deaths had occurred either on the stock trucks (3), in the sheep yards (2) or in the holding paddock after shearing (3). None of these previous sudden deaths had been investigated and the manager was advised that future ewe deaths should be investigated by the local veterinarian with tissues submitted for histopathology. Historically, Cu deficiency had been reported on these properties and cattle were routinely supplemented, but not sheep.

The MPI VS veterinarian also reported seeing a similar case of haemothorax in one cull-for-age ewe from a line of 395 from the Tararua district (outbreak 3, case number 5; Table 1). As a result of the initial investigation, a case definition of sudden death in cull ewes due to haemothorax was developed and an email alert sent over the MPI VS veterinarian network asking for further cases to be reported and samples taken. Over the following 5 weeks, a further six outbreaks affecting eight dead ewes (outbreaks 4–9, case numbers 6–13; Table 1) were reported from around the country. Fresh liver was submitted from case numbers 7–13 to Awanui Veterinary Dunedin (NZ) for estimation of liver copper concentration. Fresh pluck (lungs with the attached heart attached within an intact pericardium, and with associated mediastinal tissues) from case numbers 7–10 and fixed aorta from case numbers 11–13 was submitted to Awanui Veterinary Christchurch (NZ) for histopathological examination.

Samples of aorta from three clinically normal ewes were also taken at slaughter from three culled ewes, randomly selected during a processing day (17 March 2022) at Alliance (Dannevirke, NZ).

Clinical findings

Necropsies of the dead ewes were performed by the VS veterinarian at each abattoir. In 12/13 (92%) dead ewes, a large blood clot was present in the thorax (Figure 1) and in one case the blood clot was present in the tissues dorsal to the heart (case number 7; Table 1). In all outbreaks where material was submitted for histological examination (case numbers 7–13; Table 1) a tear in the aorta was found. In cases numbers 9–13 the tear was grossly identified at necropsy and in case numbers 7–8 the tear was identified at histology. The ewes from outbreaks 4, 5, 6, 8, and 9 all died during the belly wash procedure prior to slaughter, the ewe from outbreak 7 was condemned at arrival. Belly washing is a pre-slaughter hygiene procedure used to reduce the faecal or soil contamination of sheep prior to slaughter. The belly wash pen usually holds 40–200 sheep and the sheep are washed 0.5–24 hours prior to processing. Nozzles spray water from the floor of the pen for a duration of 100 seconds.

Table 1. Outbreaks of sudden death likely due to aortic rupture in ewes at slaughter at abattoirs in the upper North Island of New Zealand by date of notification. Each table row represents a consignment of cull ewes from a different farm of origin (an "outbreak") and the case numbers refer to the sequential number of the ewes affected.

Outbreak	Case number	Date	Farm location (district)	Sheep	Incidence (%)	Ruptured aorta diagnosed
1	1–2	25/11/2021	Gisborne	Cull ewes	2/458 (0.44%)	No
2	3–4	25/11/2021	Gisborne	Cull ewes	2/349 (0.57%)	No
3	5	16/11/2021	Tararua	Cull ewes	1/395 (0.25%)	No
4	6	30/11/2021	Taupō	Cull ewes	1/400 (0.25%)	No
5[a]	7	6/12/2021	Wairoa	Cull ewes	1/140 (0.71%)	Yes
6[a]	8	12/12/2021	Central Hawkes Bay	Cull ewes	1/351 (0.28%)	Yes
7[a]	9	12/12/2021	Ruapehu	Cull ewes	1/220 (0.45%)	Yes
8[a]	10	14/12/2021	Tararua	Cull ewes	1/182 (0.55%)	Yes
9[a]	11–13	31/12/2021	Marlborough	Cull ewes	3[b]	Yes

[a]Samples collected for pathological examination.
[b]No denominator data available.

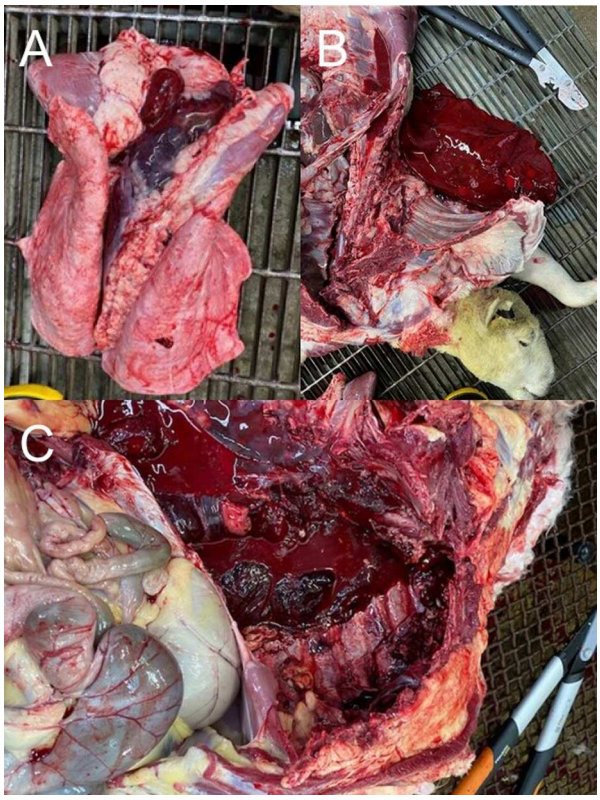

Figure 1. Post-mortem images recorded as part of an investigation into sudden death of ewes prior to slaughter at abattoirs in the upper North Island of New Zealand from three affected ewes showing examples of haemothorax found in 12/13 ewes that died during pre-slaughter hygiene preparation.

Laboratory findings

Analysis of trace element and heavy metal content in liver of affected ewes

Liver samples from case numbers 7–13 were submitted for trace element and heavy metal analysis. Liver Cu concentrations are displayed in Figure 2A. The median (min, max) liver Cu, Se and Zn concentrations were 710 (min 248, max 1,871) µmol/kg, 3,749 (min 847, max 5860) nmol/kg and 715 (min 479, max 862) µmol/kg, respectively. All mineral liver concentrations were within the normal reference range for sheep in New Zealand and there was no indication that affected sheep had concentrations outside this reference range (Grace *et al*. 2010). However, this does not exclude a period of deficiency earlier in the sheep's life that may have weakened the mechanical strength of the aortas. Three ewes (case numbers 8–10) had liver Cd concentrations of 1,100, 963, and 2,308 nmol/kg. It is currently uncertain what the Cd concentrations signify as there is no published reference range.

Analysis of Cu concentration of liver and serum of ewes on the affected farm

On 10 December 2021 10 liver biopsies and 10 blood samples were collected from similar aged ewes to case numbers 1–4 on the same station as outbreak 1, the index farm. Liver biopsies and blood samples were collected from a separate random sample of 10 ewes. Samples were submitted to Awanui Veterinary (Palmerston North, NZ) for Cu analysis. The mean liver Cu concentration was 466 (SEM 221) µmol/kg (adequate range > 95 µmol/kg), and the individual liver Cu concentrations are plotted in Figure 2B. The mean serum Cu concentration was 10.4 (SEM 0.69) µmol/L (adequate range > 8.0 µmol/L), and the individual serum Cu concentrations are plotted in Figure 2C. Again, all mineral liver concentrations were within the normal reference range for sheep in New Zealand (Grace *et al*. 2010) and there was no indication

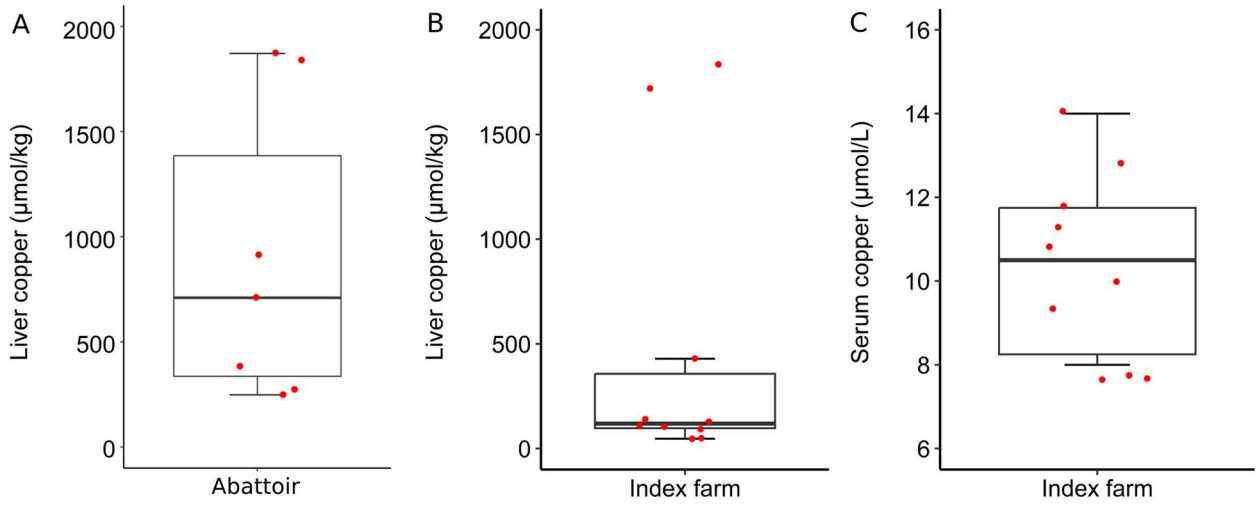

Figure 2. Box and whisker plots showing the distribution of (A) liver Cu concentration (µmol/kg) from seven cull ewes with aortic rupture that died in an upper North Island New Zealand abattoir prior to slaughter, and (B) liver Cu concentration (µmol/kg) and (C) serum Cu concentration (µmol/L) in 10 ewes of a similar age and from the index farm where the first recorded case of sudden death associated with aortic rupture was diagnosed. The bold horizontal line is the median and the upper and lower limits of the box are the upper and lower quartiles, respectively.

that in-contact sheep from the index farm had concentrations outside this reference range.

Pathological findings

The three clinically normal ewes were Romney or mixed breed sheep from three different properties in the North Island (King Country, Hawkes Bay and Tararua districts) that had no previous history of pre-slaughter death by haemothorax. Transverse sections of aorta from these normal sheep and from case numbers 7–13 were stained with H&E, Masson's trichrome, and Van Gieson's elastin stain and examined. Masson's trichrome stains collagen fibres blue, and Van Gieson's stain produces intense black staining of elastin fibres. Olympus cellSens Standard 4.1.1 imaging software (Evident Corporation, Tokyo, Japan) was used to measure the thickness of the aortic wall in the seven affected and three clinically normal ewes where histology was performed. In each of the transverse aortic sections stained with H&E, where the aorta was intact, measurements were taken at 25x magnification in 10 different, randomly selected locations. The measurements in the affected ewes were not taken at the exact locations of the tear because the haemorrhage and inflammation would have interfered. Instead, they were taken from an adjacent normal area of aorta. A summary of the histological findings and the aortic wall thickness results is shown in Table 2.

A random effects model was fitted to the aortic wall thickness data, the fitted model found that the mean aortic wall thickness in the affected ewes was 1.87 (95% CI = 1.48–2.26) mm, compared with 2.72 (95% CI = 2.12–3.31) mm for the controls, but aortic thickness measurement varied in different parts of the sections and there was overlap between the aortic wall thickness in clinically normal and affected sheep (Table 2, Figure 3A, E and I). Subjectively, there was increased collagen in the tunica media in three of the affected sheep on Masson's trichrome staining (Table 2, Figure 3F and J), and 5/7 of the affected ewes had decreased elastic fibre staining and density (Table 2, Figure 3G and K) compared to the clinically normal ewes (Figure 3B and C, respectively). Haemorrhage was seen surrounding the aorta of all affected ewes and in four ewes, haemorrhage dissected between the tunica media and the tunica adventitia (Table 2, Figure 3I, J and K).

Electron microscopy

Using a scalpel blade, a 2-mm cube of the tunica media of each aorta was removed from the paraffin blocks used to prepare the histological sections from one of the clinically normal sheep and case numbers 7–13. The paraffin was melted by briefly placing the cube in a warm (70°C) oven, then the tissue cubes were processed through four changes of xylene before being moved through decreasing concentrations of ethanol and finally placed in buffer, as described by Graham and Orenstein (2007). The cubes were post-fixed in osmium tetroxide and embedded into epoxy resin. Thin sections from the resin blocks were stained using lead citrate/uranyl acetate and viewed using an FEI Tecnai G2 Biotwin Transmission Electron Microscope (Hillsboro, OR, USA) at the Manawatū Microscopy and Imaging Centre (Massey University). Electron microscopy (EM) of the aortas of five of the affected ewes showed a decrease in recognisable elastin fibres compared to the clinically normal ewe and there was often light grey, homogenous material separating smooth muscle cells (Figure 3H and L, blue arrowhead), which was not apparent in the normal ewe (Figure 3D). In three affected sheep, the

Table 2. Aortic wall thickness and subjective changes in collagen and elastin staining observed at histological examination in seven mature cull ewes with aortic rupture compared to three clinically normal mature cull ewes, following an outbreak of sudden death in ewes at slaughter at abattoirs in the upper North Island of New Zealand.

Case number	Aortic wall thickness (mm)	Collagen morphology and staining	Elastin morphology and staining	Pathology
Clinically normal	3.0–3.7	Unremarkable	Unremarkable	NA
Clinically normal	2.6–3.6	Unremarkable	Unremarkable	NA
Clinically normal	1.8–2.1	Unremarkable	Unremarkable	NA
7	1.5–2.3	Increased collagen in the tunica media	Decreased density and intensity of staining	Dissection, haemorrhage, fibrin
8	1.8–2.5	Increased collagen in the tunica media	Decreased density and intensity of staining	Haemorrhage, fibrin, haemosiderin
9	1.6–2.6	Unremarkable	Unremarkable	Dissection, haemorrhage, fibrin
10	1.8–2.4	Unremarkable	Unremarkable	Dissection, haemorrhage
11	1.7–2.0	Unremarkable	Decreased density and intensity of staining	Dissection, haemorrhage
12	1.2–1.7	Increased collagen in the tunica media	Decreased density and intensity of staining	Haemorrhage
13	1.2–1.8	Unremarkable	Decreased density and intensity of staining	Haemorrhage

NA = not available.

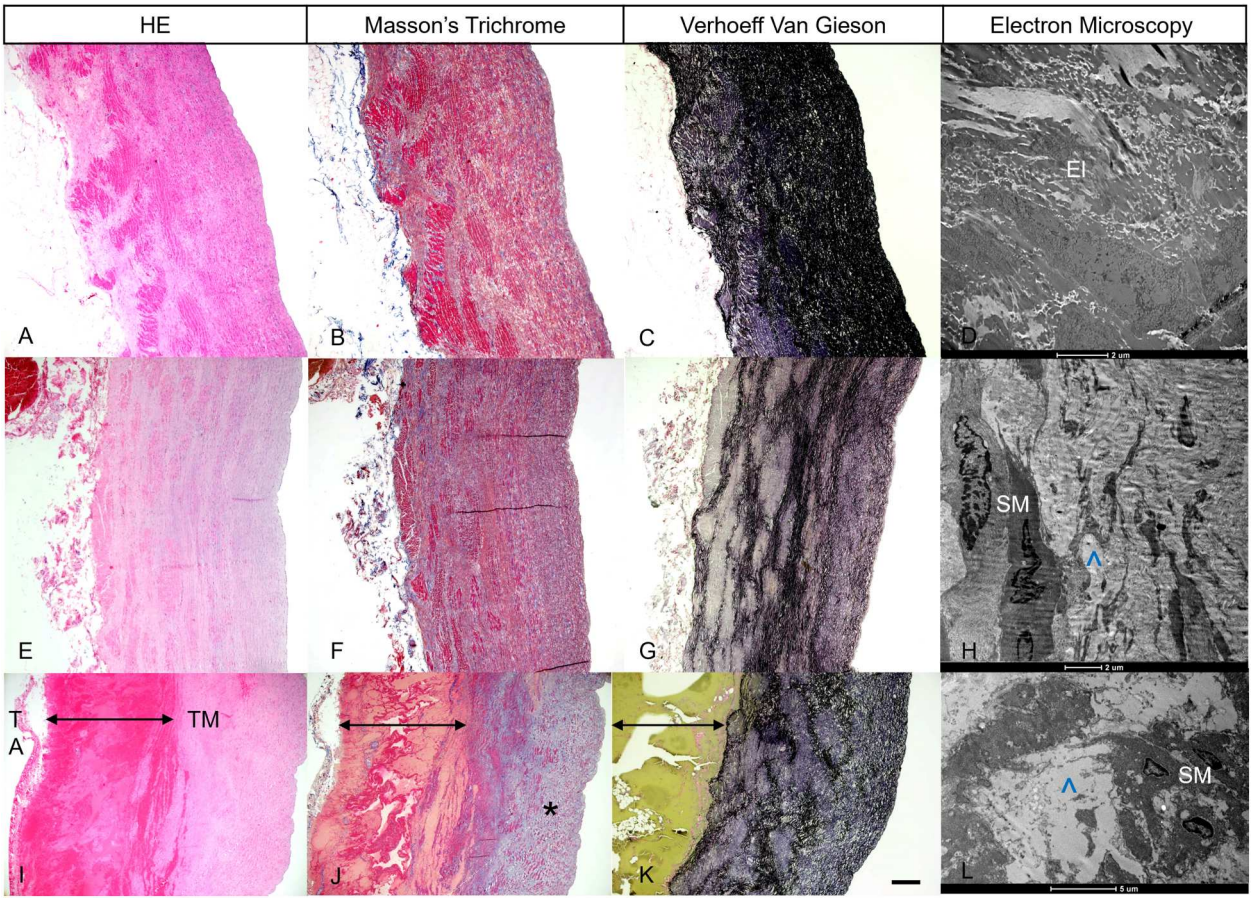

Figure 3. Photomicrographs (A–C, E–G, I–K) and electron micrographs (D, H, L) of sections of the aorta from ewes included in an investigation into sudden death associated with aortic rupture prior to slaughter. Images A, E, and I are stained with H&E, B, F, and J with Masson's trichrome, and C, G, and K with Van Gieson's elastin stain. All sections are from a similar section of the aorta with the aortic lumen to the right of each image. Images A–D are from a clinically normal ewe and show dense elastin fibres that stain black (C), and which are also seen in the tunica media in the electron microscopy (EM) image (D: dark grey, labelled EI). Images E–H are from an affected ewe and show a marked decrease in elastin, (reduced black staining in G), and in the EM image (H) smooth muscle cells (SM) are separated by pale homogenous material (^), and with no normal elastin visible. Images I–L are also from an affected ewe and show extensive haemorrhage (I, J, K: between the arrows) dissecting between the tunica media (TM) and tunica adventitia (TA) with (J) expansion of the tunica media collagen (*), and (K) reduction of elastin (black staining) compared to the clinically normal sheep, with haphazard arrangement of elastin fibres. EM (L) shows a lack of elastin, vacuoles within the cytoplasm of smooth muscle cells (degeneration) and an increased amount of light grey, homogenous material separating smooth muscle cells (^). Scale bar (K) = 100 µm and applies to all photomicrographs while for the EM images, the scale bars are shown on the image.

smooth muscle cells contained small clear vacuoles within their cytoplasm (Figure 3L), but it was not possible to determine if this was a true degenerative lesion or a consequence of formalin fixation (although these were not seen in the normal sheep). In the two affected sheep that appeared to have normal elastin density in the aorta (case numbers 9–10) there was notable disruption of the elastic laminae (Figure 4), with short, fragmented laminae arranged haphazardly.

Discussion

A short MPI-led investigation which identified 13 aged cull ewes that may have died from aortic rupture has been described. In seven cases the aortic rupture was observed, with histopathology showing that the aorta was thinner in affected ewes compared to unaffected clinically normal ewes. Further comparisons found that 3/7 affected ewes had increased collagen in the tunica media and 5/7 had decreased elastic fibre staining and density compared to three clinically normal ewes.

Aortic rupture is a rarely recorded cause of death in sheep (Souto *et al*. 2017; Bashir *et al*. 2020; Massey University data on file) and yet in less than 2 months, 13 cases were found at a small number of New Zealand abattoirs. This was clearly a most unusual and worrying finding and warranted further investigation. In the referenced studies (Souto *et al*. 2017; Bashir *et al*. 2020; Massey University data on file) the sheep deaths were on farms, whereas in this study the deaths were at the abattoir. It therefore seems likely that stress and trauma, experienced either during transportation or during handling and processing at the abattoir may have caused a possibly already thin aorta to rupture. It is notable that many of the

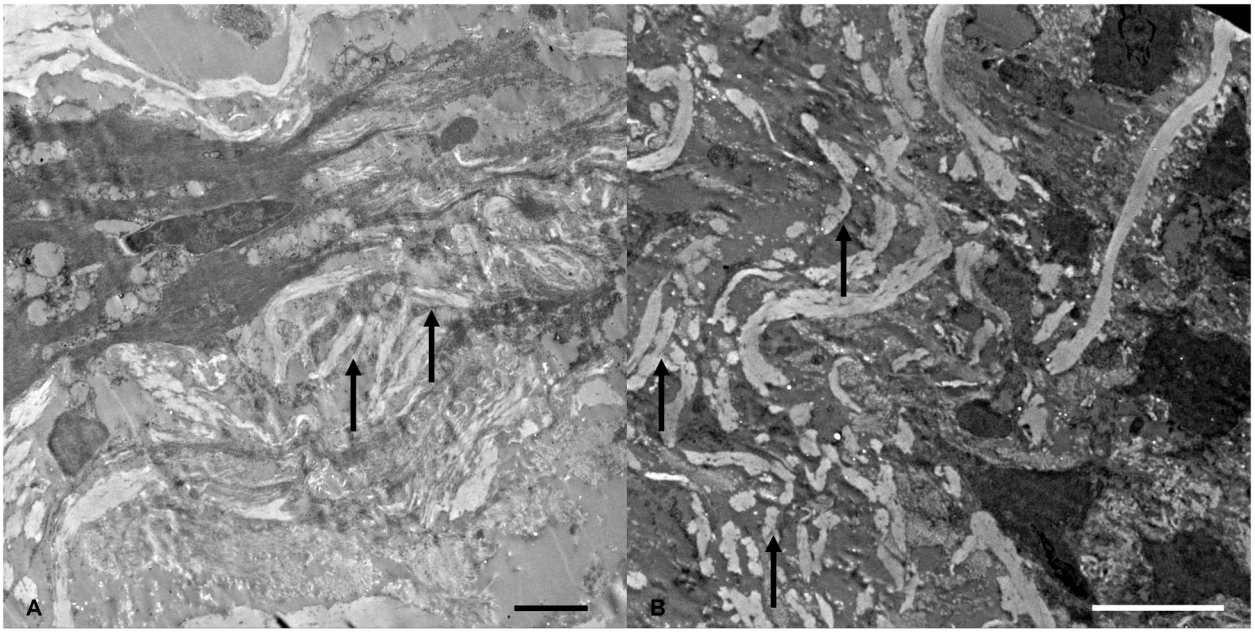

Figure 4. Electron microscopy of the aortic tunica media from two ewes dying prior to slaughter at an abattoir in the upper North Island of New Zealand and affected by aortic rupture. There is marked disruption of the elastic laminae (arrows), which are fragmented and haphazardly arranged. Scale bar in A = 2 µm, B = 5 µm.

deaths occurred during the belly wash process prior to slaughter.

Blunt force trauma is a known cause of aortic rupture in people, with bizarrely even a case recorded after a person was attacked by a ram (Živković and Nikolić 2013). However, there was little, or no subcutaneous bruising or evidence of trauma observed during the post-mortem examination of the dead ewes, which likely rules out this possible cause. It is, however, possible that poor integrity of the aortae made these sheep more vulnerable to manipulations that would not usually be expected to cause such damage or cause bruising to the carcase.

The histological examination of the aortae from ruptured and clinically normal sheep suggested the aortae from affected sheep were thinner and had decreased density and staining of elastic fibres or disruption of the elastic laminae. This fits with our working hypothesis that the aortae of these sheep were weakened, possibly through previous disruption to collagen and elastin formation, and thus more likely to rupture. In humans, the elastin content of the aorta has been reported to decrease with age while the collagen content increases (Tsamis *et al*. 2013), and increased collagen deposition has also been reported in the aortic wall in people with thoracic aortic dissections (Wang *et al*. 2006). Increased collagen was seen in three of the affected ewes in this study, and decreased elastin in five, and all were older cull ewes, so it is possible that age contributed to changes or dysregulation in the extracellular matrix of the aorta (Wang *et al*. 2006). However, ageing changes alone would not be sufficient to explain aortic rupture in these sheep, as the disease would be expected to be much more common if it was solely a degenerative, age-related change. In humans, mutations in various smooth muscle, collagen, elastin, and TGFβ pathway genes have been associated with thoracic aortic aneurysm and dissections (Pinard *et al*. 2019), and a genetic predisposition could be possible in sheep given the first four affected ewes were from the same farming operation. However, no detailed pedigree information was available to determine the relatedness of the affected animals.

It is important to note that the differences in aortic thickness found in this study could be due to sample location or tissue trimming and it would be important in the future to match the sites of measurement more accurately in clinically normal ewes to those in the affected ewes.

As well as genetic factors, dietary deficiencies could predispose to aortic pathology through effects of Cu on elastin formation and structure. Cu deficiency has been associated with aortic rupture in pigs and poultry (Graham 1977; Pletcher and Banting 1983). In particular, young pigs raised on Cu-deficient diets develop severe abnormalities of connective tissue due to defective cross-linking of collagen and elastin, as copper is required for lysyl oxidase activity, and this enzyme plays a major role in cross-linking (Hill and Davidson 1986). The samples from case numbers 7–13 and similarly aged ewes from the source farm for outbreak 1 showed that liver copper concentrations in affected sheep and clinically normal sheep were variable but all were within the published reference ranges. This indicates that Cu deficiency may not be involved in the aetiology of aortic rupture in sheep, but more work is required to be certain. It was not possible to assess cross-linking of collagen

on EM of the affected sheep in this study as the collagen content of the aortic sections that were subsampled for EM was low, and the aortic samples had been fixed in formalin and embedded in paraffin prior to EM, rather than being fixed in glutaraldehyde and processed directly for EM which yields better ultrastructural preservation (Graham and Orenstein 2007).

In conclusion, aortic rupture in older New Zealand ewes is potentially more common than previously thought. However, how prevalent and what the likely causes are, remains to be established. Currently, some data on ewe deaths at abattoirs is held but access to and publication of this could be restricted to preserve farm and processor confidentiality. Instead, a prospective longitudinal study at a sample of abattoirs with full post-mortem examinations of dead sheep needs to be implemented by VS veterinarians to get better quality data.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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