Using ultrasound to study chronic non-progressive pneumonia in lambs in New Zealand

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Report of Trial

Abstract
Chronic non-progressive ('enzootic") pneumonia is a disease with important consequences to lamb growth in New Zealand. Inability to diagnose pneumonia ante mortem is a barrier to finding cost-effective ways to control this disease in lambs in New Zealand. Overseas, researchers have used ultrasound to diagnose pneumonia in adult ewes and dairy calves. The method is cheap and non-invasive. The aim of this study was to assess whether this technique could be used to diagnose pneumonia in lambs in New Zealand. Lamb lungs were scanned by ultrasound, and their lungs were then scored at slaughter. In this study, although normal lungs were easy to identify on ultrasound, we were not able to accurately diagnose cases of lung consolidation or pleurisy. A modification of the technique used would be necessary to use the method in ante-mortem studies of pneumonia.

Introduction
Pneumonia in lambs is an extremely costly disease in New Zealand (Goodwin et al. 2008). Interventions that would decrease the incidence of pneumonia in lambs in New Zealand could potentially save farmers thousands of dollars per year. The economic effect of pneumonia has been estimated at between 32 and 78 million dollars per year not including costs due to mortality (Goodwin et al. 2008b). One study identified some management factors associated with the development of pneumonia (Goodwin et al. 2008a). However, with the exception of shearing lambs at weaning, these findings did not translate into useful management recommendations to farmers. Vaccines to prevent pneumonia have been trialled in NZ (Leigh 2011, Goodwin et al. 2004b) and overseas (Thonney et al. 2008, Lehmkul and Cutlip 1985) and found to be ineffective.

Studies on pneumonia in lambs have used gross pathology of the lung at slaughter as a measure of how badly the lamb is affected (Goodwin-Ray et al. 2008a, Baird et al. 2012). Limitations of this technique include that some pneumonia may be self-resolving (so the lack of lesions at slaughter doesn't mean the lamb didn't have a negative growth rate from pneumonia) (Harris and Alley 1977), short amount of time for visual inspection in the abattoir chain (Baird et al. 2012), and can't evaluate the progression of the disease or the effect in younger lambs (unless you kill them before they get to slaughter weight).

Pneumonia in lambs has been extensively studied in New Zealand, as it is one of the most important diseases affecting lamb growth (Goodwin et al. 2008b). Prevalence is high but mortality is low (Goodwin et al. 2008a). Chronic progressive pneumonia affects average daily growth rate in lambs (Goodwin et al. 2004a, Alley 1987) but it is not known whether this effect on growth rate occurs throughout the rest of the life of the lamb from first infection until slaughter, or only when it is acutely infected. In other words, if a lamb finisher buys lambs with already extant CNP lesions in the lungs will they grow more slowly?

The main bacteria involved are Pasteurella haemolytica and multocida (Black 1997, Black et al. 1997), but other organisms are important (Alley 2002), including Mycoplasma (Alley and Clarke 1977, Thurley et al. 1977, Pfeffer et al. 1983, Alley et al. 1999), viruses (Davies 1977, Sharpe et al. 1978), and Bordetella (Chen 1988). Heat stress is believed to be important (Black et al. 2002), as are management decisions such as shearing on the day of weaning, keeping lambs on farm longer and breeding replacement ewe lambs (Goodwin-Ray et al. 2008). Since pneumonia reduces growth rates, keeping lambs on farm longer is

likely a consequence of the disease. But practical, on-farm means to prevent pneumonia from occurring are currently lacking. If these measures were known, we don't know when they would have to be implemented (i.e. pre-weaning or at weaning or throughout the lamb's life until slaughter in autumn). And if a vaccine were developed it would be useful to be able to measure the efficacy over time from vaccination to slaughter, and to choose lambs for a vaccine trial were not already affected.

In the UK, veterinarians use ultrasound to diagnose lung pathology in sheep and cattle (Scott 2011, Scott 2014). They claim this technique is fast (as fast as pregnancy diagnosis), accurate, and inexpensive. Ultrasound has also been used to diagnose chronic enzootic pneumonia in dairy calves in Canada (Buczinski et al. 2013, Buczinski et al. 2014). This technique has been used to develop a vaccine for chronic enzootic pneumonia of dairy calves (Ollivett 2014).

If this technique could be validated on lambs in New Zealand, and if the technique is as fast and simple as the UK authors claim, it could be used to improve our understanding of pneumonia in lambs in New Zealand. This would allow researchers to find practical, on-farm methods of preventing this disease from occurring, resulting in increased lamb growth rate and better income for farmers. The aim of this study was to determine whether ultrasound could be used to diagnose pneumonia in live lambs before slaughter.

Methods

All procedures in this study were approved by the Grasslands Animal Ethics Committee (AgResearch). In April 2015, a group of 50 lambs was sourced from AgResearch Grasslands Aorangi farm in Palmerston North. Lambs were EID tagged. A rectal temperature taken, and nasal discharge was scored on a scale of 0 to 2 (0= no discharge, 1=dried crusts around the nostrils, 2= flowing mucoid discharge plus dried crusts around the nostrils). If an animal was observed to be coughing, this number was recorded. However we were unable to find a systematic way of recording coughing frequency. In 2016 the trial was repeated on 25 lambs, with modifications. Nasal discharge scores and temperatures were not taken, but EID numbers of any lambs observed to be coughing were recorded.

For ultrasound scanning, in 2015 the lamb was confined using a small portable headgate so that we could scan both sides of the thorax. In 2016 lambs were confined in a crush, positioned so the front of the crush caught them midway along the ribcage with their forelegs and head outside. A helper held the head of the lamb so that they would not move around. In both seasons, water and ultrasound gel or obstetric lubricant were applied to the bare patch of skin caudal to the elbow for ultrasound contact. Scanning was done with a DP-vet 6900 ultrasound scanner (rented from BCF Ultrasound Australasia) with a 5MHz sector probe.

The first year, the probe was held transversely to the intercostal spaces, the heart was located, and a frozen image recorded on the scanner immediately caudal to the heart (approximately intercostal space 5) dorsal and ventral, and one rib space caudal to that (intercostal space 6) dorsal and ventral. The rest of the area visible from this bare patch of skin (most of the lung space) was then scanned, including cranial to the heart, but images were not recorded. A score of 0-2 was given based on whether apparent consolidation was seen (0=no consolidation, 1=up to 50% apparent consolidation, 2=>50% apparent consolidation). Lambs were then released. In 2016 the heart was located and scans were recorded cranial to the heart to try and capture the left apical and cardiac lobes. Instead of giving a score, frozen images were recorded from four to six sites, attempting to capture most of the area cranial to the heart, dorsal and caudal to the brisket. After scanning was completed, and before slaughter, 138 captured frozen images of the scans from 24 lambs were analysed as to whether ultrasound scans had the following characteristic appearances: pleural line, 'scatter" echogenicity, consolidation and fluid. If lambs had one or more images that looked normal, the lamb was categorized as normal. Otherwise they were categorized as consolidation, pleurisy, or unsure.

On April 16 2015, lambs were slaughtered at CMP meat plant in Marton. Lungs were removed from the slaughter chain and individually bagged. Once slaughter was completed, lungs were weighed, photographed each side from the dorsal and ventral aspect using the camera from a 'Samsung Galaxy S4 mini" cell phone. Each lung lobe was evaluated for consolidation and the approximate percentage of lung affected was recorded. A different person recorded the approximate percentage lung consolidation after slaughter than had recorded the approximate consolidation by ultrasound. On April 5 2016 lambs were slaughtered at Kintyre works, Carterton. Lungs were collected and photographed as previously but not weighed. Lungs were scored by a different person than the one who had scored the ultrasound images.

Results

Lambs weighed between 32 and 51 kg liveweight. The rectal temperatures of the 50 lambs in 2015 ranged from 38.3 to 40.4 degrees Celsius. A nasal discharge score of 0 (no discharge) was recorded for only two lambs, the rest half (n=23) were recorded as a score of 1 and half (n=23) as a score of 2.
On ultrasound, in 2015, 14 out of 50 lambs had a score of 0 recorded for the left side and 11 out of 50 recorded 0 for the right side lung. In 2016, 15 lambs were categorized as 'probably normal" based on the frozen images from ultrasound. These were lambs where one or more of their four or five frozen images had the typical appearance of normal lung. There were no lambs where all of the scanned images looked completely normal (unlike the year before). One lamb was categorized as 'probable pneumonia – consolidation and fluid", many had mixed results where it was unclear whether the scan showed abnormal lung or some other tissue.

At slaughter in 2015, 30 of the 50 lambs had some consolidation visible (60%). However, only three of the right lung and five of the left lungs had any consolidation in the diaphragmatic lobes. Where lambs had consolidation this was found in the apical and cardiac lobes (left apical lobe 22% had some affected lung (between 3% and 90% of the lobe affected), left cardiac 26%, left diaphragmatic 6%, right apical 52%, right cardiac 22%, right diaphragmatic 6%, right intermediate 8%). Out of 50 lambs, 14 had one or more lobes with >20% consolidation.

Statistical analysis was not performed on these results.
At slaughter in 2016, two lambs had significant consolidation in the left apical and cardiac lobes, and four others had minor amounts of consolidation (2% of the lobe affected). Six lambs had major (>20%) and 12 minor (2-5%) consolidation on the right side. The rest were normal.
Two lambs were coughing during the time lambs were being scanned. One of these had no lung pathology at slaughter and the other only minor (2% of the right apical lobe) consolidation.
The lamb that I was convinced during scanning was normal was in fact normal at slaughter. The lamb that I was convinced I saw fluid at scanning did have pleurisy recorded at slaughter, although only minor consolidation (2% of the left apical lobe). Ten lambs were categorized as normal on the analysis of the frozen images from the scans and were in fact normal at slaughter. Three lambs had some consolidation at slaughter but were called normal on the analysis of the scan images (a type II error). These lambs had small amounts of consolidation and normal lung was seen above the consolidation. One lamb was called abnormal from the analysis of the scan images but was in fact normal at slaughter (type I error). This was likely from interpreting other tissues in the thorax as abnormal lung tissues.
Pictures of lungs and frozen images from the scans are provided as appendices (attached).
There was no correlation between the rectal temperatures or nasal discharge scores and the amount of consolidation.

Discussion

In 2015, it became clear almost immediately as lungs were being scored at slaughter that the lungs with consolidation were affected in the apical and cardiac lobes. Images had not been recorded in these areas, only in the diaphragmatic lobes. The diaphragmatic lobes were not severely affected in these lambs. For this reason a statistical analysis was not performed as it was immediately clear that the two scores would not correlate. This was not because the technique was not valid, but because the examiners believed that lambs would be more severely affected. We expected lesions similar to those seen on post mortem of lambs that had died in the field with pneumonia, in which the ventral half to 1/3 of the lungs were consolidated. Previous reports (Goodwin-Ray et al. 2004a, Baird et al. 2012) reported the most significant effect on growth rate when consolidation affected >25% or >50% of the lung surface area. In this study although some of the individual lobes were affected up to 100%, there was never more than 25% of the overall surface area of the lungs affected in any of these lambs.

Therefore the trial was repeated in 2016. Fewer lambs were scored in 2016 for logistical reasons. Attempts were made to refine the trial to make it more practical and more repeatable. Unfortunately, lambs were less severely affected in 2016.

The visualisation of the lung was easy to do and required little in the way of preparation and setup. This is in line with published reports overseas (Scott 2011). The part that was most difficult was finding the location of lung edges in the standing animal. Consolidation shows up in the most ventral part of each lung lobe, telling the difference between a thin bit of consolidation and non-lung tissues in the cranial ventral thorax was not reliable in this study. The liver extends further cranially in the standing animal than on post mortem, and in 2015 in some cases liver was recorded as consolidation. In retrospect, these could be identified as liver thanks to the portal vein (if located). Normal lung was easy to visualise (if lung had a characteristic appearance on ultrasound it was definitely normal). This suggests the technique could have some use – for example to exclude lung disease in individual adult animals as has been shown in previous studies (Scott 2011).

These issues may just have been due to inexperience with ultrasound. No specific ultrasound training was undertaken before this trial was set up. This was for two reasons – firstly because the aim was to determine whether the technique could be used by a veterinarian to diagnose enzootic pneumonia in a live lamb, not to determine whether the diagnosis could be made by a professional ultrasonographer. The second reason was because a previous trial (Buzcinski et al. 2013) showed that interobserver agreement was excellent and specialised training should not be necessary to diagnose pneumonia in calves using this technique.

It should have been easy to tell pleurisy as this would present as fluid areas beside the lung. Some lambs in this study were diagnosed at slaughter with pleurisy but this was not seen on ultrasound. It is likely that the areas of attachment of the lung to the pleura or to the diaphragm were small. Pleurisy was successfully diagnosed by ultrasound in some cases.

Why were these results inconclusive when overseas published reports have shown the technique to be so good? Previous research was done on dairy calves (Ollivett 2014, Buzcinski et al. 2013, 2014) or adult ewes (Scott 2014, 2011) or adult cattle (Scott 2013, Abutarbush 2012). I was unable to find previously published reports of this technique on lambs. It is possible that there is less lung field to see on a small lamb, between the liver caudally, the brisket ventrally and the shoulder cranially, with the heart in the middle. A more experienced ultrasonographer may have had better results with this technique, or a better method of recording where normal lung was seen may be make this technique useful.

Limitations of the technique include that it was more labour intensive and time consuming than was expected, although practice and refinement of the technique should improve this. Previous reports suggested that lung ultrasound should take less than 5 minutes in an adult ewe (Scott 2014), 10 minutes for a cow (Abutarbush et al. 2012). The first year we took almost 8 hours to scan 50 lambs, (on both left and right sides, including setting up, scoring nasal discharge, taking temperatures, tagging lambs, weighing lambs, working out how to get the rented machine to record the images, and discussing the best way to confine the lambs). The second year we scanned one side of 24 lambs through a crush crate in 2 hours. This is too slow for the envisioned application of this technique – to scan mobs of lambs several times through the year in order to explore how the lesions change in live animals.

If the technique is able to be refined so that it is effective, it could be used for a number of applications. These include studying the effect of the disease on growth rate throughout the life of the lamb, determining whether lesions resolve in some lambs by the time of slaughter, the effect of the disease on ram performance, finding the best treatment for pneumonia, the role of metaphylaxis, the role of vitamin B12, and hopefully practical methods that can be used on-farm to decrease the prevalence and impact of this disease, as well as using this technique to trial vaccines against pneumonia.

Conclusion

The aim of this trial was to validate the use of ultrasound scanning to diagnose chronic non-progressive pneumonia in lambs. In this study, we were not able to reliably categorize the degree of consolidation in lungs. Ultrasound was able to show normal lung where present, and fluid. The technique was not precise enough degree to be able to be used for studying pneumonia in live lambs.

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