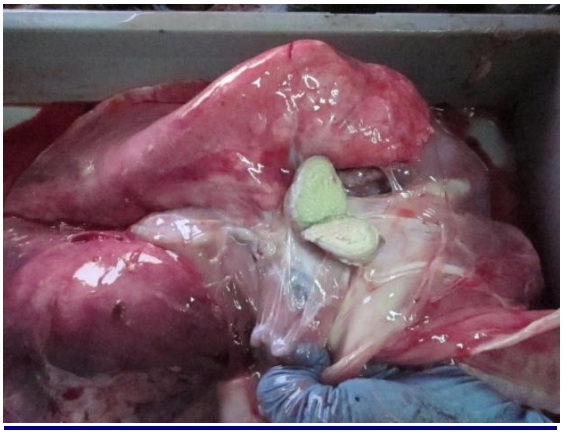
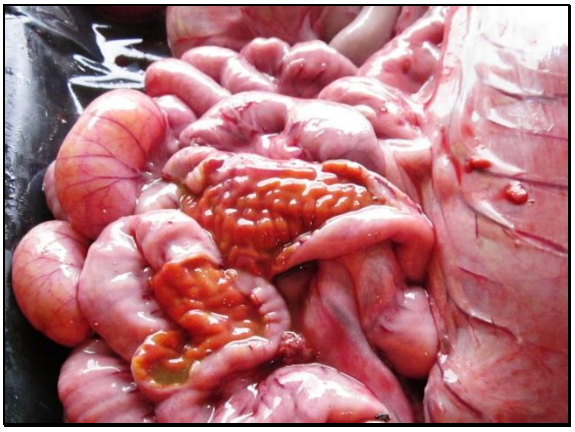
A pilot study on the value of fallen stock necropsy to sheep farmers, with emphasis on ewe mortality

EBLEX R&D Grant Final Report March 2013





F.M. Lovatt, Flock Health Ltd., Balmer House, Eggleston, Co Durham, DL12 0AN.

B. W. Strugnell, AHVLA Thirsk, West House, Station Road, Thirsk, YO7 1PZ.

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1. Introduction

Annual ewe mortality rates in the UK are estimated to range between 3% (Johnston *et al.* 1980) and 5-8% (Scott 2007) representing a loss of between half and one million ewes per year. In addition, estimated ewe replacement rates, at 20 per cent (approximately 3 million ewes per year), are influenced by longevity and may be compromised by diseases causing premature death or involuntary culling. The profile of diseases which contribute to mortality and involuntary culling in adult ewes in the UK flock is at present not known with any certainty. It is probably best estimated using Veterinary Investigation Diagnosis Analysis (VIDA) data generated by the Animal Health Veterinary Laboratories Agency (AHVLA) endemic disease scanning surveillance programme, which examines 5-700 ewes annually. Necropsies are performed at regional laboratories at subsidised rates. However, this system may have inherent biases (Nevel & Stark 2009), the sample size is small and its focus is on new and emerging, rather than endemic, disease (Gibbons *et al* 2008). There may therefore be under-representation of important endemic diseases, such as mastitis, which farmers diagnose themselves, Johnes disease and ovine pulmonary adenocarcinoma (OPA), where low economic value of affected animals often precludes diagnostic investigations. Table 1 shows the frequency of all diagnoses made on adult ewe carcases by the AHVLA scanning surveillance programme, between 2006 and 2011.

It is a legal requirement that all fallen stock from UK farms must be collected for appropriate disposal by a licensed collector. Large numbers of sheep carcases are therefore collected to one site on a daily basis and this represents an opportunity for such carcases to be examined in order to establish the cause of death. This pilot study aimed to investigate the diagnostic potential of this material, with an emphasis on adult ewes, and consider how such information might be applied to improve sheep health, welfare and productivity.

This study was confined to adult ewes because it was considered that (a) they were likely to be under-represented by diagnostic interventions commonly performed and (b) identifying causes of ewe mortality offers much scope to improve productivity by focusing interventions to mitigate these losses

	2006	2007	2008	2009	2010	2011
No Diagnosis	58	62	43	76	94	53
Parasitic Gastroenteritis	57	49	82	94	109	41
Chronic Fluke	19	24	92	116	58	16
Acute Fluke	15	31	66	67	28	5
Listeria encephalitis	39	23	33	36	34	25
Pneumonia NOS	24	23	25	33	28	18
Mannheimia	27	28	21	22	26	14
Haemonchosis	16	21	22	20	13	5
Caseous lymphadenitis	20	22	20	7	10	1
Pregnancy Toxaemia	17	13	17	17	16	12
HypoCalcaemia	14	22	12	17	14	13
Acidosis	10	8	14	15	14	7
Dental Disease	11	9	16	9	3	3
Johnes	8	11	16	17	12	6
Trauma/Fracture	16	11	15	15	12	11
Copper Toxicity	9	13	15	11	12	7
Malnutrition	9	5	14	12	7	4
Cerebro-cortical necrosis	5	6	14	4	9	9
Ovine pulmonary adenocarcinoma	9	15	8	21	10	19
Parasitic pneumonia	12	8	5	18	4	6
Torsion/ Redgut	6	1	6	12	10	5
Cl. perfringens type D	13	9	10	11	0	7
Metritis NOS	10	9	9	9	9	6
Dosing Gun Injury	14	8	8	10	16	7

Table 1. Frequency of the commonest diagnoses made after necropsy of adult ewes by (AH)VLA, 2006-2011. Carcase submissions only (*i.e*. tissues and other samples not included) NOS – not otherwise specified

2. Materials and Methods

Eleven necropsy sessions were undertaken at a large fallen stock collection centre (FSCC) in the north east of England, and necropsies performed on an average of 10 adult ewe carcases during each session. All carcases were examined anonymously, with no clinical history and no knowledge of the farm of origin. All necropsies were performed by the authors. Carcases which were externally grossly autolysed or decomposed were avoided. For each carcase examined, the breed, estimated age, body condition, degree of autolysis, gross post mortem findings and suspected diagnosis, were recorded. Where a diagnosis could not be made on the basis of gross necropsy alone, further laboratory-based diagnostic testing was performed. Diagnoses were made according to predefined diagnostic criteria (see table 2).

3. Results

Approximately 70 ewes arrived at the centre from 1430 and 1900 each day. Many of these were severely autolysed and were not included in the study. Further ewes in which the cause of death was obvious (*e.g.* dystocia, prolapsed uterus, vagina or intestines or limb fracture) were also not included.

106 ewes were necropsied, all by the authors, with some assistance from practitioners from Castle Veterinary Surgeons, Barnard Castle on some occasions. Overall, a firm diagnosis was reached in 75 of 106 carcases (70%). However, some diagnoses were considered incidental, such as salmonellosis due to *Salmonella* 61:K:1,5,7, and insufficient as the sole cause of death.

A diagnosis considered sufficient to account for death was reached in 68% of cases (table 2). Nine of the 106 carcases (8.4%) were too autolysed for meaningful evaluation, despite pre-screening to avoid these carcases. In 20 of 106 carcases (19%), no diagnosis was reached despite, in some cases, the presence of lesions that may have been categorised as pathological.

In a small number of cases, the suspected diagnosis could not be confirmed by further laboratory testing (*e.g.* suspect psoroptic mange, larval paramphistomum infection), which may have been the result of a treatment, of which the investigators had no knowledge.

3.1. Sample population data

Age and breed information for the ewes necropsied are shown in figures 2 & 3. The sample population probably matched the local ewe breed distribution.

As expected, the majority of the ewes had full or broken mouths (figure 3), but 23/106 (21.6%) were 6 tooth ewes or younger, representing a significant economic loss to the owners of these animals.

Diagnosis	Frequency	Diagnostic criteria & Notes
No diagnosis	20	
Autolysis	9	Carcase too autolysed for meaningful diagnosis
Mastitis	11	Gross udder appearance only (4/11) +/- bacteriology: contaminants (6/11) ; *M. haemolytica* cultured (1/11)
Acute fascioliasis	8	Gross pathology only
Pasteurella bronchopneumonia	7	Gross pathology only (2/6) + bacteriology (contaminants 2/7) or histopathology (4/7)
Chronic suppurative pneumonia	6	Gross pathology only (6/6)
Neoplasia	6	Gross pathology only (3/6) or histopathology (3/6)
OPA	6	Gross pathology + histopathology (6/6)
Johnes	6	Gross pathology + faeces PCR (6/6)
Metritis/ dystocia/ obstetrical	4	Gross pathology only (4/4)
Abomasitis	2	Gross appearance + negative WEC (2/2) Note: Suspected type II telodorsagiosis or recent PGE & anthelmintic treatment
Chronic fascioliasis	2	Gross pathology only (2/2)
PGE	2	Gross pathology + WEC (2/2)
Poor Dentition	2	Gross pathology only (2/2)
Peritonitis	2	Gross pathology only (2/2)
Acidosis	2	Gross pathology + rumen pH <5.5 (2/2)
Salmonella 61:K;1,5,7	2	Gross pathology (scour) + Salmonella cultures (2/2) Note: Not considered an adequate cause of death
Dosing gun injury	2	Gross pathology only
Megaoesophagus	1	Gross pathology only
CLA	1	Caseous mediastinal lymphadenopathy + *C. pseudotuberculosis* cultured
Endocarditis	1	Gross pathology only
Acetonaemia/ Fatty Liver	1	Gross pathology + ketonuria
Abdominal torsion	1	Gross pathology only
Chronic nephritis	1	Gross pathology + histopathology
Suspect larval paramphistomum	1	Gross pathology only
Total	106	

Table 2: Results and diagnostic criteria

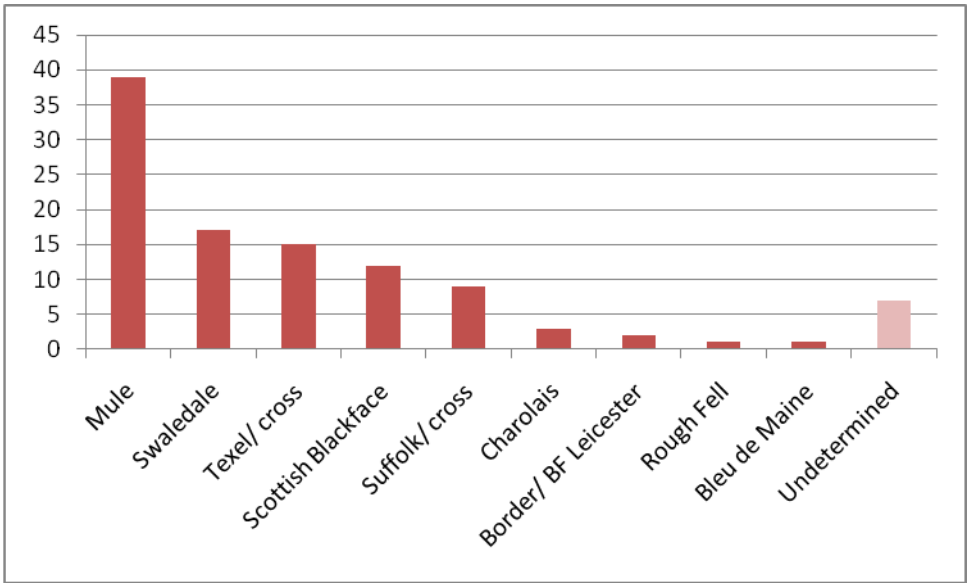

Figure 1. Frequency of each breed in the sample population (Note: In some cases heads were missing so breed couldn't be determined)

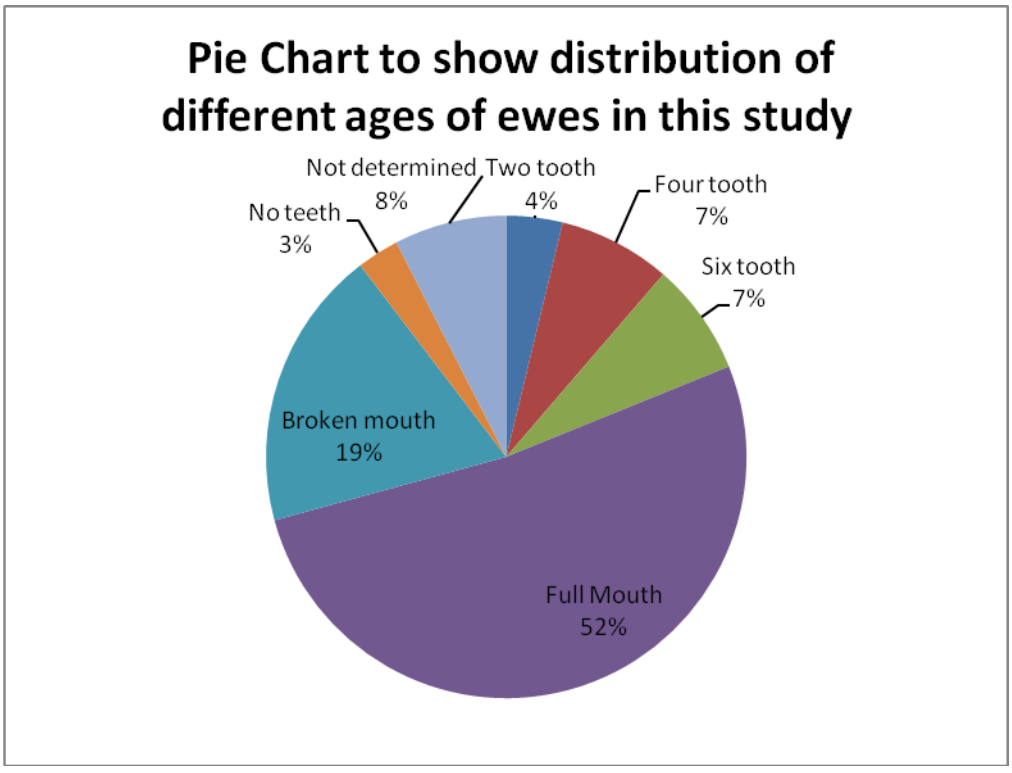

Figure 2. Age distribution of ewes in the sample population

4. Discussion

4.1. Mastitis

Figure 3. Gross appearance of ovine udder with acute mastitis from this study

Eleven of 106 ewes (10.3%) were diagnosed with acute mastitis, making it the most frequent positive diagnosis. Of these eleven cases, bacteriology was performed in eight, resulting in coliforms and/or *Proteus* in all but 1 case, where *Mannheimia haemolytica* was cultured (details in Appendix A). In two cases, there was concurrent evidence of *Pasteurella* septicaemia (figures 4a & b); such laryngeal lesions are rarely reported with mastitis.

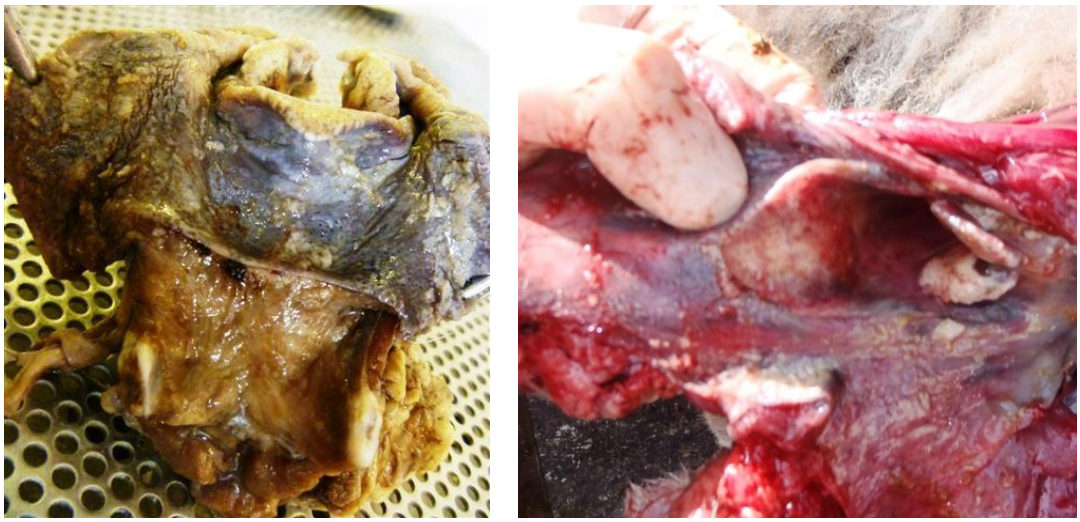

Figure 4a & b. Formalin-fixed and fresh laryngeal lesions in a ewe with acute mastitis. These lesions may be a result of *Pasteurella* septicaemia.

All swabs for bacteriology were taken after searing surfaces and charcoal swabs were used to minimise growth of contaminants. Possible reasons for apparently disappointing bacteriology results are suggested in the constraints section. If the identification of the causal organism was considered important in future fallen stock based surveys of ovine mastitis, efforts would have to be made to perform clean culturing techniques, selective culture techniques to avoid contaminants and prompt plating of swabs.

Accurate estimates of the true prevalence of ovine mastitis in UK sheep are lacking. It might be expected that the current surveillance arrangements might under-estimate prevalence, because the diagnosis is obvious and will in most cases be made by the farmer alone. This contention is supported by VIDA data, which recorded only 120 diagnoses of ovine mastitis between 2006 and 2012, of which 31 were from carcases. A third of these cases were due to *M. haemolytica,* a third due to *Staphylococcus aureus* and a third due to other organisms. The within-flock annual incidence of ovine mastitis in the UK has been estimated to range from 1-15% (Winter 2001) and it has been further suggested that the UK industry loses 7-12% of the breeding ewe population as a result of mastitis (http://www2.warwick.ac.uk/fac/sci/lifesci/research/greengroup/sheepmastitis/udderconformation/ accessed on 1/3/2013). While prevalence is likely to be highly variable between flocks, it is important to quantify national losses and fallen stock surveys may prove a cost effective method of achieving this. Further, if sampling techniques could be optimised, such an approach could enable collection of bacterial isolates for research or to provide antimicrobial sensitivity data to inform treatments.

Accurate within-flock incidence data, such as that collected as part of a systematic fallen stock necropsy-based diagnostic service, could inform cost-benefit analysis of interventions such as the improvement of lactating ewe nutrition.

4.2 Fasciolosis

Acute liver fluke was diagnosed as the cause of death in eight ewes (7.5%), though the first case was not observed until October. This is an example of a disease for which prompt diagnosis in fallen stock, as gross necropsy is pathognomonic, could lead to timely intervention and disease control at the farm level. In practice, this may mean that an effective treatment is given after the first ewe dies, rather than after the tenth; a significant economic saving. Fluke was estimated to have cost the GB sheep industry £13-15 million in

2011 (EBLEX 2012), and there is good evidence to suggest that prevalence has increased since then.

Fascioliasis is also a good example of a disease whose geographical footprint has expanded over recent years, probably as a result of animal movements and climate/ habitats which favour the parasite and its intermediate host. Routine systematic necropsy of fallen stock could detect other such changes in a timely fashion, resulting in raised awareness among farmers and more appropriate action. The spike of acute fluke cases was very pronounced. This information could be extrapolated to local cattle herds, *i.e* treatments for chronic fluke (the only life stage for which there is a treatment licenced for lactating cows), can be correctly timed based on the known likely first ingestion of metaceceriae.

Chronic fascioliasis was seen in two cases, but there was evidence of healed/ treated disease in two others. This manifestation of fascioliasis is crucial to diagnose as it can have severe impacts at lambing time by increasing periparturient metabolic disease, and reducing lamb size/ viability, colostrum quality and subsequent milk yields.

4.3 Bacterial bronchopneumonia

Pasteurella bronchopneumonia accounted for 7 of 106 cases (6.6%) (details in Appendix A). The death of adult ewes due to *Pasteurella* pneumonia is useful information for the flock, as preventative interventions in the form of vaccination are available.

Again, in this study, bacteriology was rather unrewarding but the gross appearance of lungs with this condition is obvious, and the diagnosis is readily confirmed histologically, as it was in five cases in this report. As stated above, the vaccinal history of the necropsied ewes was unknown. A high within-flock incidence of P*asteurella* bronchopneumonia in adult ewes in the face of vaccination should lead to investigations into vaccine storage and administration, and thereafter into potential predisposing factors, such as trace element deficiencies, nutritional factors or immune-modulatory infections such as Border disease.

4.4 Chronic suppurative pneumonia

Chronic suppurative pneumonia was diagnosed as the cause of death of six ewes (5.6%). Infection may be introduced by a number of different routes, such as poor needle hygiene or rough clipping, and infection may become established if other factors, such as nutrition or trace element status, are suboptimal. Hence, it is important for the farmer to know if

chronic suppurative pneumonia is making a significant contribution to within-flock ewe mortality, as remedies are available with appropriate veterinary collaboration.

4.5 Tumours/ Neoplasia

Neoplasia was diagnosed as the cause of death in six ewes (5.6%). While there are some well-recognised predisposing causes of neoplasia in sheep, such as bracken toxicity, tumours are generally seen as one-off events with little relevance to the rest of the flock. In a previous study on ten Scottish sheep farms, neoplasia was responsible for less than 1% of ewe deaths (Johnston *et al.* 1980) though this study suggests that the prevalence in this population was much higher.

4.6 Ovine Pulmonary Adenocarcinoma (OPA), Jaagsiekte

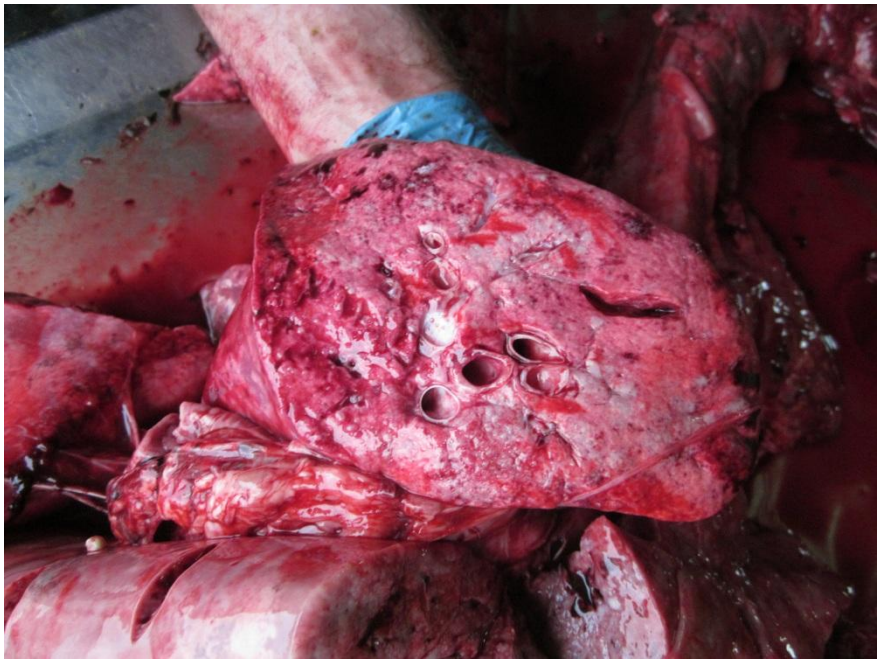

Fig 5 OPA lesion (confirmed histologically) in a ewe necropsied as part of this study

Six of 106 ewes (5.6%) were diagnosed with OPA as the cause of death in this study (details of gross pathology and histopathological findings in Appendix A).

Estimates of the true mortality attributable to OPA in the UK ewe flock are difficult to ascertain as there remains no reliable diagnostic test in the live animal, and dead or cull ewes are unlikely to be necropsied due to their low economic value.

AHVLA scanning surveillance diagnosed 138 cases of OPA in the six years between 2006 and 2011, averaging 28 cases per year (two per regional laboratory per annum) over the whole of England and Wales of which a variable number were from whole carcase submissions (table 1).

This is estimated to represent around 0.2-0.65% of all diagnosable submissions (Griffiths *et al* 2010) and is certainly a significant underestimate of the prevalence of OPA mortality in the UK flock, due to biases in this sample. This study has demonstrated that fallen stock material *is* diagnostically useful for this disease on gross pathology with confirmation by histopathology. More accurate prevalence data would be obtainable through larger fallen stock necropsy work.

From an individual flock perspective, knowing that a significant proportion of adult ewe mortality is attributable to OPA may or may not be useful information. A vaccine is neither available nor in prospect in the short or medium term. Current advice to control the disease includes the quarantine of bought-in stock, consideration that the virus may remain infectious in contaminated areas and on equipment for several weeks and age-stratified management of the flock (Cousens *et al* 2009). More radical interventions such snatching lambs from dams at birth (Voight *et al* 2007) and the use of embryo transfer (Parker *et al* 1998) have been suggested but are probably only appropriate for high value pedigree flocks. A programme of avoiding breeding replacements from older ewes or at least, confirmed cases of OPA would seem intuitively sensible but, to date no published work has evaluated this approach.

It is likely that OPA is a significant constraint to production in high-prevalence flocks. Accreditation schemes, for flocks which sell breeding sheep, do not currently exist but would be very useful, particularly if aimed at the grandparent level of the UK sheep stratification system. Such schemes would need to be based on examination of fallen and cull ewes and would require careful consideration of exactly what was being assured, but the benefits to buyers of such sheep (*e.g.* replacement mule shearlings), could be considerable.

Wider application of necropsy of fallen sheep, if coupled with clinical information, would identify high prevalence flocks on which interventions could be trialled.

4.7 Johnes Disease

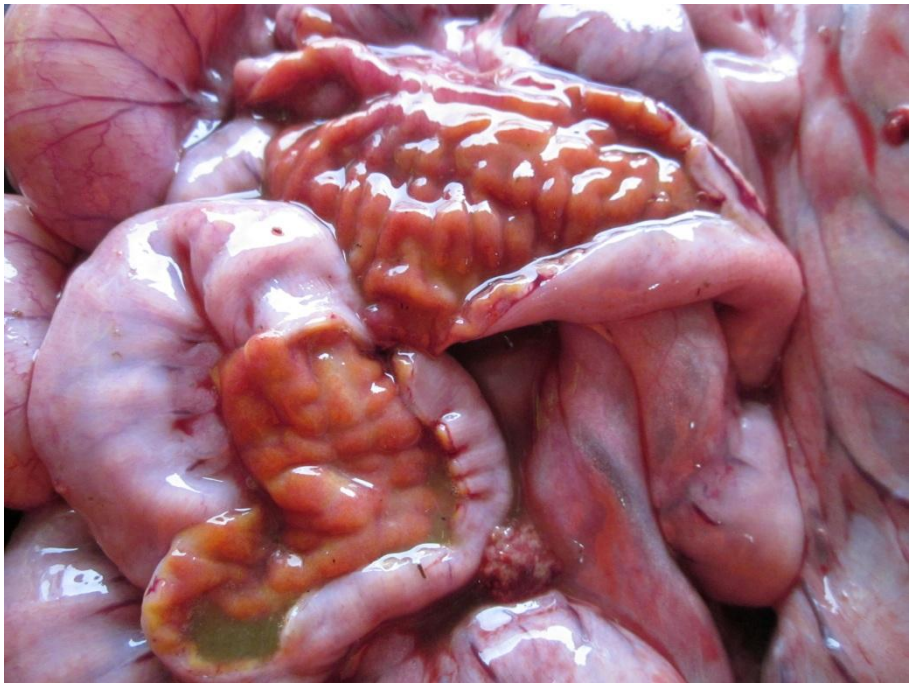

Fig 6 Multibacillary Johnes Disease in a ewe necropsied as part of this project (confirmed by faecal PCR)

Johnes disease was diagnosed in 6 of the 106 ewes (5.6%). The diagnosis was confirmed in all cases by a positive PCR test on intestinal contents coupled with consistent gross post mortem findings. Of the six PCR positive cases, small intestinal mural thickening with yellow discolouration (figure 6), characteristic of the multibacillary form of Johnes (Clarke & Little 1996), was observed in only two ewes. Gross *post mortem* findings in other positive ewes comprised mild intestinal thickening, enlarged mesenteric lymph nodes, ascites, emaciation and sometimes scour, the latter probably attributable to concurrent parasitic gastro-enteritis (PGE). There is a risk of false positive results with the faecal PCR test because it can detect microbial DNA just 'passing through' the GI tract and not causing disease. In these cases, there was evidence of hypoproteinanemia and enteric pathology, making the diagnosis likely, but some caution may be required in general when using this test, especially where carcases are not available. Histopathology of appropriate tissues (mesenteric lymph nodes and jejunum, would be required if a high level of certainty is sought. As is the case for OPA, the prevalence of Johnes disease in the UK sheep flock is likely to be significantly underestimated by the available measures. Scanning surveillance made 259 diagnoses of Johnes disease in sheep from throughout England and Wales in the period 2006-2011 (70 from carcase submissions).

Johnes disease probably contributes significantly to productivity losses on high prevalence farms and interventions to mitigate its impact are reasonably well documented. These include the avoidance of breeding flock replacements from known positive animals, trying to minimise exposure of young lambs to older animals excreting the organism, and vaccination. If the within-flock prevalence could be estimated, costs of disease and therefore cost-benefit analysis of any interventions could be assessed.

4.8 Caseous Lymphadenitis (CLA)

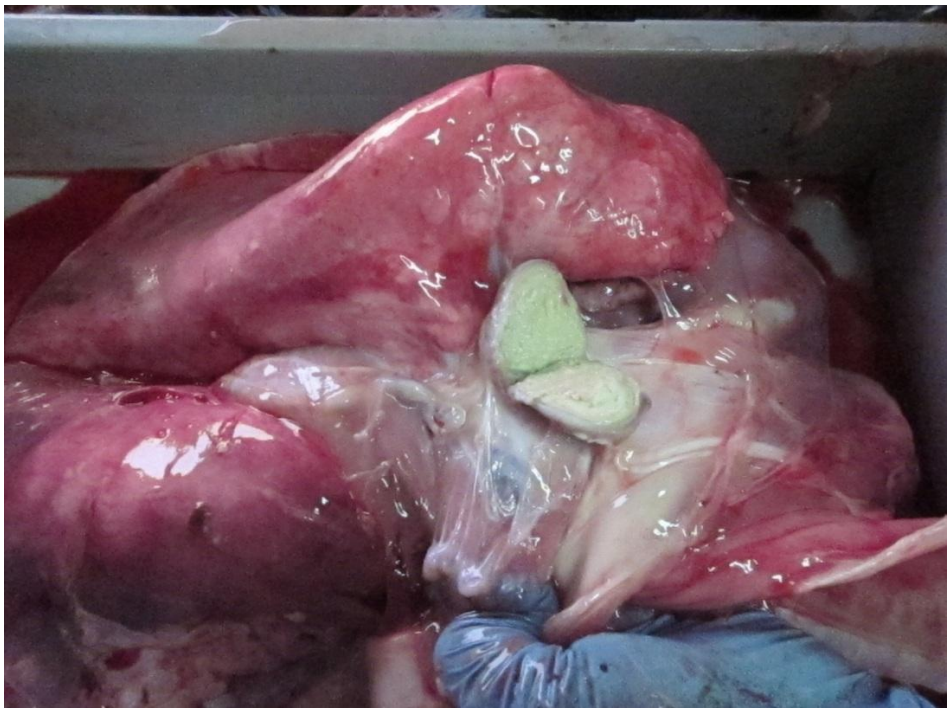

Fig 7 CLA lesion in a mule ewe necropsied as part of this study

CLA was diagnosed in one ewe. Serological tests for CLA have moderate sensitivity and specificity, and culture of suspect gross lesions is the gold standard. However, there are implications to the sampling of abscesses on farm in the possible spread of the disease to cohorts. This is an important and high-profile disease though there is continued reluctance amongst farmers to acknowledge its presence within a flock. Flocks that undertake an additional level of surveillance for CLA through a survey of fallen stock could provide extra assurance to purchasers of breeding stock.

4.8. Cases where no diagnosis was made

Tables 6a & b in Appendix A summarise the 20 cases where no diagnosis was made. In some cases, clinical history would have been helpful as it may have directed suitable further

testing (*e.g.* if neurological signs were shown the brain would have been removed and cultured/ examined histologically). It is possible that, with clinical history, a diagnosis would have been made in some of these cases. However, we considered that it was not a good use of resources to remove and process the brain for histopathology/ cultures in all cases. In some cases, there was gross evidence of hypoproteinaemia (body cavity effusions, gelatinous atrophy of fat), with no infectious cause identified. Poor nutrition would be a possible cause in those cases, which is useful information for farmers. Costs of further testing were reasonably high in cases where no diagnosis was made, which may itself be useful information for farmers aiming for cost-effective spending on diagnostics; it may be more prudent to take and retain tissues and monitor further mortality (the next mortality may present a clear diagnosis), than to process all tissues for one carcase in which the cause of death is unclear. There should be scope for this in any future fallen stock-based diagnostic service.

It can be seen from tables 6a & b that diagnoses were not made, even if suspected, where there was insufficient evidence. This is important as interventions can be expensive and require time and effort, so must be based on accurate diagnosis.

One important finding was that in three cases, clostridial enterotoxaemia was suspected from the gross findings but could not be confirmed. This may suggest that diagnostic criteria which are applicable for diagnosis of clostridial disease in lambs, may not be suitable for ewes, or that it is difficult to confirm this diagnosis in this age of animal. Again, vaccination on suspicion may be a better way to spend money that pursuit of a further unlikely-to-be successful /conclusive laboratory testing.

4.9. Further laboratory testing.

Whether or not further confirmatory diagnostic testing is required to make a diagnosis is a balance between the level of confidence one requires to be sure that the diagnosis is correct, and the costs of confirming a suspected diagnosis. For example, in this study, we confirmed all cases of OPA and multibacillary Johnes disease histologically and by PCR respectively, but in both cases, the gross appearance is almost pathognomionic so it could be argued that money is better spent on intervening to remedy problems rather than on 'gold-plating' a fairly secure diagnosis.

Table 7 (Appendix A) summarises the cases where a diagnosis was made in this study (77 cases), and assesses the need for further diagnostic testing in each case under each level of diagnostic 'certainty'. If a 'working' hypothesis is required, then a diagnosis could be made without recourse to further testing in 85.8% of cases (62% overall). If on the other hand one requires more certainty, then only 53.3% of positive cases can be diagnosed on the basis of gross necropsy alone. These numbers are useful markers when planning any necropsy-based diagnostic service in which further testing is not included in the cost of the necropsy. It is reasonable to assume that it is up to whoever is paying for further testing, to decide, with appropriate veterinary advice, whether or not it is required

In the case of carcases where no diagnosis was made, more further laboratory testing was performed in search of a diagnosis than in cases where no diagnosis was made, as one would expect. It would be unwise to infer too much from these results because in practice, all further testing would be informed by clinical history and discussions between farmers and their advising veterinary surgeons.

4.9. Costs

4.9.1 Time performing necropsies.

On average, each ewe necropsy, including sample collection, recording of findings and cleaning tables between carcases, took around 30 minutes and cost £73.20, including rent at the FSCC, veterinary time and all further diagnostic testing. That figure comprises £41.2 (56.4%) veterinary time, £22.60 (30.8%), and £9.4 (12.8%) (figure 8). It is likely though that the 30 minutes of veterinary time could be reduced as some time was spent waiting for carcases to arrive and preparing the post mortem area at the beginning and end of sessions.

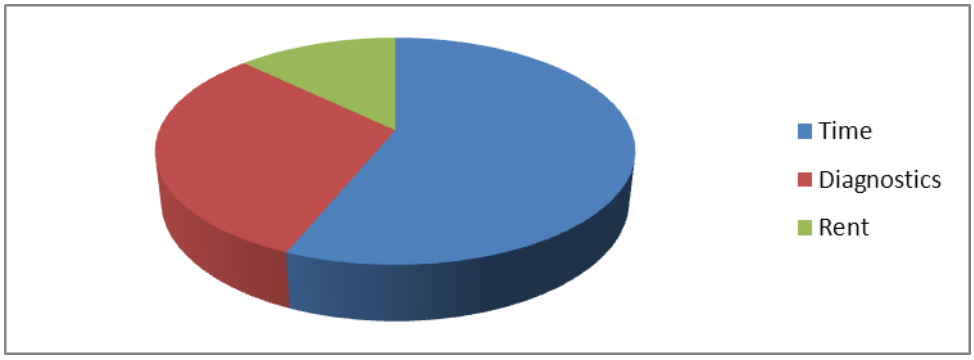

Figure 8. Breakdown of costs between veterinary time, further testing and rent at FSCC in ewe necropsy.

A figure of around £50 (£41.2 + £9.40) for gross necropsy, to include rent and other overheads but not further laboratory testing and excluding collection/ disposal, would, we consider, be a realistic balance between farmer affordability and perception of value for money. However, if a permanent post mortem room could be established at a large FSCC, we consider that economies of scale and time efficiency could probably reduce this figure. It is also a realistic figure based on estimated annual sheep flock spend on veterinary advice and medicines of between £3.50 and £7 per ewe (Scott at al 2007, EBLEX Business Pointers 2012)

5. Constraints

Some analysis of the possible limitations of this approach is appropriate. Possible limitations are discussed below:

5.1. Bias

When selecting carcases to necropsy, carcases were avoided when the cause of death was immediately obvious, *e.g.* intestines prolapsed through vulva or dystocia, on the assumption that these carcases would not be presented for post mortem examination by the farmer as the cause of death was already clear. Carcases which were clearly severely autolysed (green, emphysematous) were also avoided. This may have led to an under-representation of diseases where rapid post mortem autolysis is a feature *e.g*. clostridial disease. However, the diagnoses even of those diseases would have been difficult to make unequivocally in those circumstances, and it is probably likely that clostridial diseases in adult ewes are not as common as they are in lambs (Johnston *et al* 1980, VIDA data).

5.2. Limitations: Bacteriology

Bacteriology in this study proved unsatisfactory. Despite taking care to swab surfaces aseptically (searing surface prior to swabbing), and the use of charcoal swabs, many of the culture plates were overgrown with *Proteus* and other contaminants. This was probably due to a number of factors:

- Contaminated environment. It is possible that the problem would be partly mitigated if necropsies were performed in a dedicated and regularly disinfected post mortem room.

- Microbial ecology. Bacteria cultured after death may be opportunistic secondary invaders and thus may differ from those causing initial lesions.
- Delayed culture initiation. Most necropsy sessions took place on a Thursday, so swabs were returned to AHVLA on the Friday from where they were sent to AHVLA Bury St. Edmunds to initiate cultures on the Saturday. They would be read for the first time on the following Monday, allowing time for *Proteus* and other contaminants to outgrow pathogenic organisms.
- Prior antimicrobial administration. As the ewes were necropsied blind, there was no information as to whether antimicrobials had been administered. Clearly these would have inhibited bacterial growths

It would be prudent to investigate selective culture techniques aimed at minimising *Proteus* and other contaminant growth, whilst not inhibiting the growth of target organisms. Such techniques and media, such as cled agar, are available and should be evaluated. This would facilitate the collection of bacteria responsible for diseases such as mastitis, CLA or bronchopneumonia, should that be necessary for further molecular studies.

5.3. Cross contamination

Several carcases are collected in one wagon which means that there is some scope for cross contamination between carcases. In this study, most of the diagnoses were made would not have been affected by cross contamination because they did not concern bacterial cultures or PCRs for which cross contamination would have been important. PCR is a very sensitive technique, and we used this to confirm suspicions of Johnes disease. In all but one case, however, diagnoses of Jones disease were made on different occasions, making cross contamination impossible. On the occasion where two diagnoses of Johnes were made in one session, the sheep were consecutive so cross contamination should be considered as a possibility. However, in both cases, gross findings were consistent with a severe protein-losing enteropathy and the diagnosis was expected. It is possible that both carcases originated from the same farm, a possibility which would be known about were clinical history available. The two cases of Salmonellosis due to *Salmonella* 61:K;1,5,7 were diagnosed on the same session, so similar comments apply.

Samples were taken from within body cavities (*e.g.* faeces taken directly from colon/ rectum), and gloves were changed between every carcase, to minimise the risk of cross

contamination. In summary, while we consider that no cross contamination occurred during our trial, we acknowledge that anyone engaged in necropsy of fallen stock and diagnosing bacterial diseases using sensitive methods, should be alert to the possibility and use supplementary testing (*e.g.* histopathology, ZN smears of mesenteric lymph nodes) wherever possible, to confirm diagnoses in the minority of cases where it might have influenced results.

6. Conclusions

Although good bacteriology results have proved difficult to obtain, this study has shown that it is possible to diagnose the cause of death from fallen stock.

We propose that the systematic necropsy of fallen stock would lower the threshold for diagnostic investigation and thus improve the application of best practice on UK sheep farms.

Whether used as a tool for national disease surveillance or at the farm level, ongoing use of this concept may prove invaluable for the sheep and other livestock industries and the scope for its development is considerable.

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Appendix A Gross necropsy findings, histopathology and bacterial results

Ewe ID	Date diagnosed	a) Breed b) Estimated Age c) Degree of Autolysis d) Body Condition	Gross post mortem findings	Bacterial culture results
1.1	20/04/2012	a) Mule. b) Full Mouth. c) Moderate. d) Good-fair	Foul smelling gangrenous mastitis. Excessive purulent material within the teat system on one side. Scant colon contents - blood stained gluey. Purple diffuse discolouration of lungs. Involuted uterus.	Not done
2.6	11/05/2012	a) Mule. b) Full mouth. c) Moderate to Severe. d) Good.	Marked haemorrhage around udder with marked purulent mastitis in teat system. Scant contents of small intestine. Fibrinous pleuritis.	Not done
2.10	11/05/2012	a) Swaledale. b) Full mouth. c) Moderate to Severe. d) Fair.	Severe mastitis with purulent deposits in the teat system.	Mixed mucoid coliforms, and *Serratia* spp.
3.6	22/06/2012	a) Mule. b) Full mouth. c) Moderate – Severe. d) Fair.	Severe mastitis of one side (purulent). Watery milk with clots in. Slight fatty liver. Multifocal lung abscessation.	Scant mucoid non haemolytis coliforms overgrown with *Proteus*
3.9	22/06/2012	a) Mule. b) Broken mouth. c) Moderate. d) Fair.	One side of udder lumpy and swollen. Multifocal white lesions throughout the liver about 2 – 3 cm in diameter. Severe necrotic diphtheritic laryngitis including laryngeal ventricles and epiglottis with a pseudo membrane. Also ulcerative oesophagitis and multiple focal white lesions (15) around chest wall.	Profuse almost pure growth of *Mannheimia haemolytica*
4.1	29/06/2012	a) Mule. b) Full mouth. c) Mild to Moderate. d) Fat.	Petechiations over ventral abdomen and udder skin. Severe purulant mastitis. Purple discolouration of caecal serosa and dark red/black/purple content. Small pyogranulomatous abscesses throughout the lungs poorly encapsulated especially caudodorsal regions. Pleuritis cranially.	Non haemolytic coliforms ovewrgreown with *Proteus*
4.2	29/06/2012	a) Mule. b) Broken mouth. c) Moderate. d) Moderate – Severe.	Mastitis with serous fluid and haemorrhages in milk. Liver was fatty and greasy. There were petechial haemorrhages around the heart base and inside the thoracic cavity.	Non haemolytic coliforms overgrown with *Proteus*
5.1	20/07/2012	a) Texel/ Lleyn. b) Full mouth. c) Mild – Moderate. d) Fair.	Impression of jaundice from conjunctival mucous membranes. Severe acute mastitis with a swollen lumpy udder. Orange discolouration of the liver. Fibrinous proliferation in the laryngeal ventricle. Some small abscesses in lungs.	Mixed non haemolytic coliforms
5.3	20/07/2012	a) Mule/Texel cross. b) Full mouth. c) Moderate – Severe. d) Good – Fair.	Severe purulent mastitis. Necrotic yellow deposits in the lateral ventricles of the pharynx extending 5cm into the oesophagus.	Not done
5.11	20/07/2012	a) Suffolk cross. b) Six tooth. c) Mild – Moderate. d) Good.	Inspissated beige non-smelly material in teat cyst? Just dried off. Small rumen and purple abomasal mucosa.	Non haemolytic coliforms overgrown with *Proteus*
6.11	23/08/2012	a) Mule b) Six-tooth c) Moderate d) Fair	One side of udder distended, the other had milk present. Extensive cellulitis up to 5 cm thick in subcutaneous tissues of the udder on cut surface. Marked axillary caseous lymphadenitis. Numerous renal infarcts (old)	Mixed coliforms

Table 3. Summary of mastitis cases with bacterial culture results when available

Ewe ID	Date diagnosed	a) Breed b) Estimated Age c) Degree of Autolysis d) Body Condition	Gross post mortem findings	Histopathology
1.1	17/02/12	a) Mule b) Full Mouth c) Moderate d) Fair	Cranioventral purple consolidation affecting 40% of lung fields. Markd enlargement of the tracheobronichial lymph nodes. Excessive white froth in trachea	Multifocal coalescing areas of necrosis bordered by an intense inflammatory reaction predominantly consisting of neutrophils and streaming oat cells. Bacterial colonies can be seen scattered throughout the tissue. There is alveolar oedema present as well as multifocal areas of haemorrhage. Airway epithelium is totally autolysed.
2.11	11/5/12	a) Mule. b) Broken mouth. c) Moderate. d) Good.	Colostrum present in udder. Cranioventral purple discolouration and consolidation of 30% of the lung fields.	Widespread inflammatory reactions with neutrophils and streaming oat cells, around airways. Alveolar oedema associated with these. Some haemorrhage.
3.2	22/06/12	a) Mule. b) No teeth. c) Moderate. d) Fair – Good.	Enlarged liver with indurated regions near margins and grey discolouration. Some calcified lesions throughout liver. Purple consolidation of cranioventral lung regions (35% of lung volume).	Moderate autolysis. Airway-centred inflammatory cell infiltration which is predominantly neutrophilic in origin and overspills into surrounding alveoli. Alveolar oedema and intense alveolar septal congestion. Occasional multifocal areas of mild haemorrhage are present mainly subpleurally. In one section early streaming oat cells can be identified.
5.8	20/7/12	a) Mule. b) Full mouth. c) Mild. d) Good – Fair.	Pale conjunctival mucous membranes. Fatty Liver. Formed faeces. Cranioventral consolidation of lung fields affecting 20% of the lungs with healed fibrosis.	-
6.1	22/8/12	a) Mule b) Broken mouth c) Moderate to severe d) Poor	One eye had been scavenged. No fat in subcutaneous tissues. Large gall bladder. Patchy arborising stiff beige lesions comprising 60% of the left middle lung lobe and the right cranial lung lobe. Some inspissated froth in the airways	Severe autolysis, however, evidence of multifocal areas of bacterial colonisation surrounded by severe inflammatory infiltrates predominantly neutrophilic with some streaming oat cells seen. There is alveolar oedema associated with the affected tissue in which large numbers of pulmonary macrophages can be seen.
6.12	22/8/12	a) Mule b) Full mouth c) Moderate to severe d) Fair	Greasy white/pale liver. Cranioventral consolidation affecting 25% of lung fields, bronchopneumonia	-
10.1	29/11/12	a) No head. b) No head. c) Moderate. d) Fair.	Multifocal beige solid heavy regions throughout the lungs.	Severe inflammatory infiltrates in multifocal coalescing distribution. These tend to be expansile with necrosis present in the central areas. There are mainly degenerating neutrophils and streaming oat cells around the edge of these areas. Large numbers of parasitic forms present throughout the section. There is severe serofibrinous exudation throughout the sections and also serofibrinous expansion of alveolar septae. There is some evidence in patchy distribution of Type 2 pneumocyte proliferation and there is also smooth muscle hyperplasia dispersed randomly throughout the section.

Table 4: Summary of results for cases of bacterial bronchopneumonia

Ewe ID	Date	a) Breed b) Estimated Age c) Degree of Autolysis d) Body Condition	Gross Findings	Histopathology
1.3	17/2/12	a) Mule. b) Full Mouth. c) Moderate. d) Good-fair	Solid white lung consolidation 40% mainly caudal lobes	Severe autolysis; however, there is evidence of widespread Type 2 epithelialisation of alveoli and large foamy macrophage type cells. The affected alveoli form an almost glandular type of structure when large areas are affected.
3.4	22/6/12	a) Blue Faced Leicester. b) Full mouth. c) Moderate. d) Poor.	Extensive pleuritis with solid grey/white areas in cranioventral regions of lungs.	Mild to moderate autolysis is present. There are large multifocal expansile areas of altered pulmonary architecture with tubules and acini of cells supported on a thick fibrovascular network. The cells are cuboidal or columnar and replace normal alveolar cells. Large macrophages are present around the affected areas in very high numbers. There is a peribronchiolar lymphoplasmacytic infiltrate and quite marked fibrosis amongst the abnormal tissue.
4.9	29/6/12	a) Mule. b) Four tooth. c) Mild – Moderate. d) Good – Fat.	White homogenous material comprising 30% of lung volume and radiating out from the left middle lobe of which almost all was obliterated. Associated pleurisy.	Large areas of tissue with altered architecture showing expansile areas of tubules and aciniined by cuboidal to columnar epithelial cells. Large numbers of macrophages are present in these areas. Thick fibrovascular stroma supporting tubules and acini. One section shows pronounced lymphoplasmacytic peribronchiolar cuffing.
6.3	22/8/12	a) Mule b) Full mouth c) Moderate to severe d) Poor	Small rumen. Cranioventral 30% of the lung fields obliterated by solid white homogeneous heavy material. There was some white mottling in the caudodorsal regions too. Tracheobronchial lymph nodes enlarged	In three of the sections autolysis is so severe that cellular definition cannot be ascertained. In one of these sections there is a large focal necrotic mass bordered by indistinct inflammatory cells and some haemorrhage. The fourth section is slightly better preserved and shows severe congestion of all tissues. There are multifocal areas of Type 2 pneumocyte proliferation forming acini with large numbers of foaming macrophages in the surrounding alveoli.
8.1	18/10/12	a) Mule. b) Full mouth. c) Moderate – Severe. d) Fair.	OPA lesions comprising white homogenous solid change affecting 20% of the lungs especially in the right hand side near the hilus and the dorsal regions of the right middle lobe.	Advanced post mortem change. Extensive lining of alveoli/alveolar ducts by tall columnar cells is discernible. Although marked post mortem change is present, the findings are compatible with OPA.
10.4	29/11/12	a) Scottish Black Face. b) 6 tooth c) Moderate. d) Poor.	Lungs affected with arborising beige discolouration and very solid lung consolidation. This affected 60% of the lungs in a cranioventral distribution. There was an impression of the rib remaining on the pleural surfaces.	Examination of this tissue shows severe widespread alteration in tissue architecture. There are tubules and acini throughout the section lined by cuboidal Type 2 pneumocytes. There are large foamy macrophages scattered throughout the there is a secondary bacterial problem resulting in severe predominantly neutrophilic and streaming oat cell inflammatory cell infiltration. There is widespread congestion with some haemorrhage and widespread smooth muscle hyperplasia

Table 5. Table 4: Summary of results for cases of ovine pulmonary adenocarcinoma

Ewe ID	Gross Findings	Further testing			Notes
1.5	Widespread oedema in subcutaneous tissue. Large increase in peritoneal fluid with some fibrin.Faecal balls. One single lamb *in utero*.	None			Could have done Johnes PCR but no mesenteric lymphadenopathy. Consider poor nutrition clinical history required
1.8	There was an excess of pleural fluid in the pleural space. Prescapular lymph nodes were enlarged on the right hand side.	None			Consider clostridial enterotoxaemia but insufficient evidence
2.1	The liver was greasy. The rumen was full. There was multifocal erythema of the abomasal mucosa.The large intestine contained scant liquid contents.	WEC-Negative			Possible treated PGE clinical history required
2.4	Haemorrhagic small intestinal contents. Faeces loose. Excessive gelatinous pleural effusion and pericardial effusion. Uterus one third closed down, non smelly contents.	Glucosuria-Negative	CP toxins-Negative	Brain histology Negative	Clostridial enterotoxaemia suspected but couldn't be confirmed
2.13	Green gut contents in airways and green discolouration to lung. All else unremarkable.	Aq Hum Ca-Normal	Lung culture-*E.coli*		Possible aspiration due to hypocalcaemia (unsuccessful treatment?) clinical history required
3.10	Orange/yellow discolouration of liver. Fibrinous pericarditis and dark grey discolouration of kidney.	Worm Egg Count- Negative			Possible PGE but recently treated clinical history required
3.11	Petechial haemorrhages sclera and epicardium. Bloody gelatinous material around muscles of neck. Formed faeces but gastrointestinal tract relatively empty.	None			Possible trauma clinical history required
4.3	Serosanguinous ooze in subcutaneous tissues. Haemorrhagic/fibrinous contents of small intestine. Gluey contents distal colon. Diffuse mottling of lungs with no consolidation. Engorged meningeal vessels	Brain listeria cultures	CP toxins-Negative	Brain histology Negative	Clostridial enterotoxaemia suspected but couldn't be confirmed
4.8	Serosanguinous ascitic fluid in abdomen. All other systems unremarkable	None			?Poor nutrition
5.7	The small intestine was subjectively rather thickened with mucoid content. Mesenteric lymph nodes were enlarged.	None			Johnes PCR indicated but faeces lost

Table 6a. Gross post mortem findings and results of further testing in cases where no diagnosis was reached.

Ewe ID	Gross Findings	Further testing		Notes
5.9	The animal was poor and small. The small intestine was yellow, faeces were formed.	None		No lymphadenopathy ?Poor nutrition
6.6	Haemorrhagic duodenum and ileum wall. Formed faeces in rectum	Microscopic examination of duodenum for larval paramphistomes		Suspect larval paramphistomum but couldn't confirm
6.7	Excessive fat deposition subcutaneously and in the liver. Haemorrhagic lung	None		?
7.2	Pronounced foot rot both front feet. Calloused knees. All else unremarkable.	None		Cause of death unclear
7.7	No gross findings except scour.	Worm Egg Count- Negative		?Recent anthelmintic treatment
9.5	Liver was diffusely pale. Brown/red contents of abomasum. Lungs were pale. Evidence of blood aspiration. Blood in trachea.	Worm Egg Count- 450 epg		Suspect prepatent haemonchosis or recent anthelmintic treatment
10.3	Excessive fat all regions, otherwise no gross lesions	None		? Excessive condition
11.2	Multifocal beige irregular lesions liver capsule. Excess fluid in pleural space, Gelatinous pericardial effusion. 1 month gestation singleton *in utero*	CP toxins- α only	Histopathology- parasitic bronchitis and moderate cholangiohepatitis	Suspect clostridial enterotoxaemia but couldn't confirm
11.3	No subcut fat. Generalised gelatinous effusion body cavities. Enlarged mesenteric LNs	Johnes PCR-Negative	WEC-Negative	? Poor nutrition
11.6	Purple conjunctival mucous membranes. Healed fluke lesions liver. Purple discolouration and oedema cranioventral 30% of lung tissue	Histopathology-severe pulmonary congestion and moderate oedema		?

Table 6b. Gross post mortem findings and results of further testing in cases where no diagnosis was reached.

Diagnosis	Frequency	No. cases in this study where further testing would (not) have been required if:			
		'working' diagnosis		Gold standard diagnosis	
		Yes	No	Yes	No
Mastitis	11		11	11	
Acute fascioliasis	8		8		8
Pasteurella bronchopneumonia	7		7		7
Chronic suppurative pneumonia	6		6		6
Neoplasia	6		6	6	
OPA	6		6	6	
MB Johnes	2		2	2	
PB Johnes	4	4		2	
Metritis/ dystocia/ obstetrical	4		4		4
Abomasitis	2	2		2	
Chronic fascioliasis	2		2		2
PGE	2	2		2	
Poor Dentition	2		2		2
Peritonitis	2		2		2
Acidosis	2		2		2
Salmonella 61:K;1,5,7	2	2		2	
Dosing gun injury	2		2		2
Megaoesophagus	1		1		1
CLA	1	1		1	
Endocarditis	1		1		1
Acetonaemia/ Fatty Liver	1		1		1
Abdominal torsion	1		1		1
Chronic nephritis	1		1	1	
Suspect larval paramphistomum	1		1	1	
Totals	77	11	66	36	41
%		14.2%	85.8%	46.7%	53.3%

Table 7, showing the requirement for further (confirmatory) laboratory testing at two levels of diagnostic criteria. NB. PM-side tests (e.g. glucosuria, rumen pH, ketonuria) are not included in further laboratory testing.

Tests	Number	Unit Cost	Total		Time	Number	rate	Total
Tissue Bioc	1	38.05	38.05		BS Time	118	18.35*	2165.3
Johnes PCR	13	29.1	378.3		FL/CVS Time	11	200**	2200
BDV PCR	1	17.5	17.5					
Haemonchus diff	1	39.45	39.45					
Routine culture	22	18.15	399.3					
WEC	20	12.4	248					
Salm culture	2	14.3	28.6					
CP Toxins	3	23.45	70.35					
Bacti ID	1	46	46					
Bioc	1	7.25	7.25					
Tissue copper	3	44.1	132.3					
toxo IFAT	1	20.8	20.8					
Histopathology	25	38.15	953.75					
Listeria culture	1	17.5	17.5					
Totals			**2397.15**					**4365.3**
Rent				**1000**				
Grand Total (necropsies only)					**7762.45**			
Cost/ ewe	73.2		22.6	9.4				41.2

Table 8. Summary of laboratory costs and veterinary time spent during the project.
*Rate per 15 minutes. Therefore, 29.5 hours of necropsy time in total spent on project by BS
**Rate per session (which averaged 2.68 hours). Total necropsy time spent by FL/CVS= 29.5 hours.
BS= Ben Strugnell FL=Fiona Lovatt, CVS= Castle Veterinary Surgeons.