



Descriptive analysis of ovine mortality in sentinel sheep flocks in Ireland

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Abstract

Background Studies of sheep mortality or cause-specific mortality, in Ireland or internationally, are relatively scarce but are important in presenting baseline levels and changing trends of endemic disease. This study assessed sheep mortality and cause-specific mortality in 33 sentinel sheep flocks in Ireland.

Methods Sentinel flocks were requested to submit carcasses of all sheep that died to the regional veterinary laboratories (RVLs) of the Department of Agriculture, Food and Marine during a calendar year (2016). Postmortem examinations were performed on 1247 submissions to Athlone, Kilkenny and Sligo RVLs.

Results The median overall submission rate was 13.8 per cent (range 2.5 per cent–35.8 per cent) per adult female sheep in the flock in January 2016. The median fetal, perinatal, lamb and adult submissions per adult female sheep in the flock in January 2016 were 2.1 per cent (0.0 per cent–15.2 per cent), 3.5 per cent (0.0 per cent–20.0 per cent), 3.0 per cent (0.0 per cent–12.4 per cent) and 2.8 per cent (0.8 per cent–7.1 per cent), respectively. The frequency of detection of categories of postmortem diagnoses in fetuses, perinates, lambs and adults are presented.

Conclusions Comparisons with existing passive surveillance findings reflect some differences in the relative frequency of detection of certain categories of disease suggesting that sentinel flock surveillance could usefully supplement existing passive animal disease surveillance activities for ovine disease.

Introduction

Sheep production is a significant component of Irish agriculture and is worth approximately €250 million in terms of sheep meat output to the Irish economy annually.¹ While sheep populations are more densely

concentrated on the western seaboard, where mountain breeds are generally favoured to suit the terrain, the majority of the Irish national sheep flock of 3.87 million sheep are lowland, or lowland-crossbreeds (55 per cent), with an average flock size of 108 sheep.²

The regional veterinary laboratories (RVLs) are a network of government-funded, strategically located, veterinary diagnostic laboratories serving the farming community and fulfilling a role in generating primarily passive veterinary surveillance data. Each RVL has a catchment area which typically comprises five surrounding counties from which voluntary submissions are made by farmers on referral by their private veterinary practitioners to the RVLs. Approximately 3000 sheep carcass submissions (including approximately 1000 fetus submissions) are submitted annually to the six RVLs. The findings of these gross postmortem examinations, and ancillary tests, are reported to the referring veterinarian and are aggregated, analysed and reported to stakeholders on a quarterly and annual basis.³

Passive surveillance systems have certain recognised limitations including the potential for bias by factors which influence submission behaviour such as distance

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from the laboratory,⁴ cost of analyses⁵ or the severity of the disease outbreak.⁶ Furthermore, they rely on a 'pyramid of scrutiny' where animal owners must maintain sufficient vigilance to observe ill or dead animals and must also seek professional assistance for clinical diagnosis.⁷ Therefore an accurate assessment of ovine mortality rates or ovine cause-specific mortality will require an alternative approach. Sentinel surveillance is a form of active surveillance in which data are collected from selected, targeted groups or networks covering a subset of the population.⁸ While active surveillance systems provide an opportunity to validate passive surveillance findings, they typically require a significant investment of time and resources and therefore are often sustained for only a limited period of time.⁹

Studies examining the ovine mortality rate and, in particular, cause-specific ovine mortality in Irish flocks are lacking. Such data are important in facilitating the analysis of disease trends in the national sheep population, in determining baseline levels of endemic disease and in informing improvements in sheep husbandry and welfare to the benefit of the industry. The aim of this project was to describe the mortality and cause-specific mortality in a pilot sentinel study of Irish sheep flocks over a calendar year.

Materials and methods

Study population and enrolment

Three RVLs (Athlone, Kilkenny and Sligo) selected lowland sheep flocks, using convenience sampling, within their catchment area for enrolment in the study beginning on January 1, 2016. Thirty-three sheep flocks were selected based on their involvement in Teagasc (Teagasc is a semistate organisation responsible for research and development, training and advisory services in the agrifood sector) supervised sheep discussion groups, location, size (preferably 80–400 ewes) and willingness to participate. On enrolment, all 33 flocks completed a questionnaire requesting details on flock size, lambing pattern, feed type, disease history, vaccination protocol and mineral supplementation history. Data collected on the number of adult female sheep in each flock in January 2016 were used as the denominator for submission and mortality rate calculations for those flocks. Repeated collection of data or samples from selected sites was achieved by the agreement of all enrolled flocks to submit all carcasses of sheep (including fetuses) from their flocks to the local RVL for postmortem examination from January 1, 2016 to December 31, 2016. RVL staff routinely collected carcasses from many flocks, where distance or workload prevented farmer attendance at the RVL, to ensure that all carcasses were received for examination. Postmortem examination fees were waived for all submitted carcasses. Separately, serological screening of flocks was also offered for free to participants, during the month of

January, to facilitate those who wished to monitor their vaccination or pathogen exposure status. A maximum of 20 ewes per flock were screened for antibodies to specific abortifacients (Border disease, bovine virus diarrhoea, Q fever, salmonellosis, Schmallenberg virus (SBV), toxoplasmosis and enzootic abortion of ewes (EAEs)), the results of which are also presented here.

Postmortem examination

On receipt at the RVL, all carcasses were logged on the Laboratory Information Management System (LIMS) database which allows collation of postmortem findings with results of laboratory analyses. To minimise interoperator variation, sheep carcasses were subjected to a standardised postmortem examination with sampling (for bacteriology, molecular detection of pathogens, histopathology, toxicological and biochemical assays) undertaken in each case as appropriate and at the discretion of the pathologist. Cobalt and copper levels (in liver tissue) and selenium levels (in kidney tissue) were measured in all animals older than two months of age.

Ovine fetuses (and the placenta, if available) were swabbed for *Toxoplasma gondii* and *Chlamydophila abortus* PCR testing before removal from the bag in which they were submitted to avoid potential cross contamination. Sterile aliquots of fetal abomasal contents were aspirated for bacteriological culture, fetal brain and placenta were harvested for histopathology and fetal pleural fluid was collected for virus and antibody detection.

Histopathology

All tissue samples harvested for histopathology were fixed in 10 per cent neutral-buffered formalin for four days and embedded in paraffin wax; tissue was then cut with a microtome, stained with haematoxylin and eosin and, when indicated, additional sections were specially stained (eg, Gram stain, Congo red stain, and so on).

Bacteriology

All samples harvested for bacteriological culture were cultured aerobically on blood agar, McConkey agar and xylose lysine deoxycholate agar at 37°C for 48 hours. fetal abomasal contents were also cultured on charcoal cefoperazone deoxycholate agar under microaerophilic conditions (7 per cent CO₂) at 42°C for 48 hours. Anaerobic culture (on blood agar in an anaerobic atmosphere generation system at 37°C for 24–48 hours) of tissues was not routinely performed except at the request of the pathologist.

Additional diagnostic methods specific for abortions

C abortus real time quantitative PCR (qPCR) targeting the *ompA* gene (Primerdesign genesig kit) was employed on fetal swabs for the identification of *C abortus* DNA.

The primers and probe have 100 per cent homology with a broad range of clinically relevant reference *C abortus* sequences.¹⁰ *T gondii* real time qPCR was performed on fetal swabs as described by Saad *et al.*¹¹ Reverse transcriptase PCR (RT-PCR) analysis to identify pestiviruses (bovine virus diarrhoea and Border disease virus (BDV)) in pleural fluid was performed as previously described.¹² Real time RT-qPCR detecting an 88 bp fragment of the S3-segment of the SBV genome was also performed on tissue as previously described.¹³

Parasitology

Faecal parasitological analyses, when requested by the pathologist, were performed using fluke egg sedimentation for *Fasciola hepatica* and *Calicophoron daubneyi* eggs,¹⁴ fluke larval backwash procedure for *C daubneyi* larvae¹⁵ or McMaster salt flotation.¹⁶

Serological analyses

Serological analyses, using commercially available ELISA kits, to detect antibodies to SBV (indirect SBV antibody kit, Idexx Laboratories, USA), *Coxiella burnettii* (Q fever indirect ELISA, IDvet, France), BDV (indirect ELISA, SVANOVA Biotech, Sweden), *C abortus* (indirect ELISA, IDvet, France) and bovine virus diarrhoea (indirect ELISA, IDEXX Laboratories, USA) were performed in accordance with the manufacturer's instructions. Serological analysis to detect anti-*T gondii* antibodies was performed using a latex reagent agglutination assay for the semiquantitative determination of antibodies (Mast Group, UK) while standard agglutination tests for O and H *Salmonella* species somatic antigens was performed using a commercially available kit (Widal test, Remel Europe, UK) in accordance with the manufacturer's instructions.

Data analysis

An 'adult sheep' was defined as an ovine animal ≥ 12 months of age while a 'lamb' was the term used for ovine animals > 2 days, but < 1 year, of age. A 'perinate' was the classification given to lamb submissions between day 130 of gestation and 48 hours after birth and a 'fetus' was the designation given to all aborted lambs < 130 days of gestation. As precise dates of conception were not available, the designation between 'fetus' and 'perinate' was somewhat arbitrary and determined by the pathologist based on visual assessment of the lamb. For serological analysis, a seropositive flock was defined as a flock with one or more seropositive animals for the specific disease of interest. A carcase or fetal submission in which 'No diagnosis' was recorded did not have a proven cause of death following detailed analysis while a submission in which 'a pathogen was not identified' often had a diagnosed cause of death recorded but the causal pathogen was not discovered. Data were retrieved from LIMS and collated and analysed using Microsoft Excel 2007. Frequency

analysis was performed on the data to determine the central tendency, dispersal of the data and the relative frequency of individual diagnosed causes of death. Individual diagnoses were further categorised into broad categories of disease based on the organ system affected. As clostridial diseases constitute a commonly recognised group of diseases, with varied but distinct epidemiologies and aetiopathologies, against which health interventions such as vaccination are often employed, it was decided to categorise them separately from the other broad categories of disease.

Results

Flock profiles

Thirty-three lowland sheep flocks were enrolled in the study, with a satellite distribution surrounding each RVL as presented in [figure 1](#). The median flock size was 195 sheep (range 70–810). Twelve farms were engaged solely in sheep production while a further 21 were also engaged in cattle production. The majority of the flocks was scheduled to begin lambing in March (22 flocks) with lesser numbers commencing lambing in the earlier months of December (1), January (4) and February (6).

Vaccination status was based solely on the flock owner's declaration. Multivalent clostridial vaccination of the flock was widely practised (31 flocks) with vaccination against *Mannheimia haemolytica* and *Bibersteinia trehalosi* jointly (23 flocks) and vaccination against *T gondii* (16 flocks) undertaken at lesser frequency. Vaccination to protect against EAEs and contagious pustular dermatitis (Orf) were undertaken by five and two flocks, respectively. Only one flock did not undertake any vaccination against endemic disease. The animal-level and flock-level seroprevalence for specific abortifacient agents are presented in [table 1](#).

Submission patterns

The median number of submissions per flock during the study period was 29 (range 3–113). The median overall submission rate per adult female sheep in the flock in January 2016 was 13.8 per cent (range 2.5 per cent–35.8 per cent). The median fetal, perinatal, lamb and adult submission rates per adult female sheep in the flock in January 2016 were 2.1 per cent (0.0 per cent–15.2 per cent), 3.5 per cent (0.0 per cent–20.0 per cent), 3.0 per cent (0.0 per cent–12.4 per cent) and 2.8 per cent (0.8 per cent–7.1 per cent), respectively. The number of total sheep submissions and age-specific sheep submissions received from the 33 participant flocks during 2016 expressed as a percentage of the total number of adult female sheep in the flock in January of that year are presented in [table 2](#).

The temporal distribution of ovine submissions during the study period is presented in [figure 2](#).

'No diagnosis' was recorded in 5.0 per cent of adult sheep submissions, 5.9 per cent of lamb submissions and 5.3 per cent of perinate submissions.

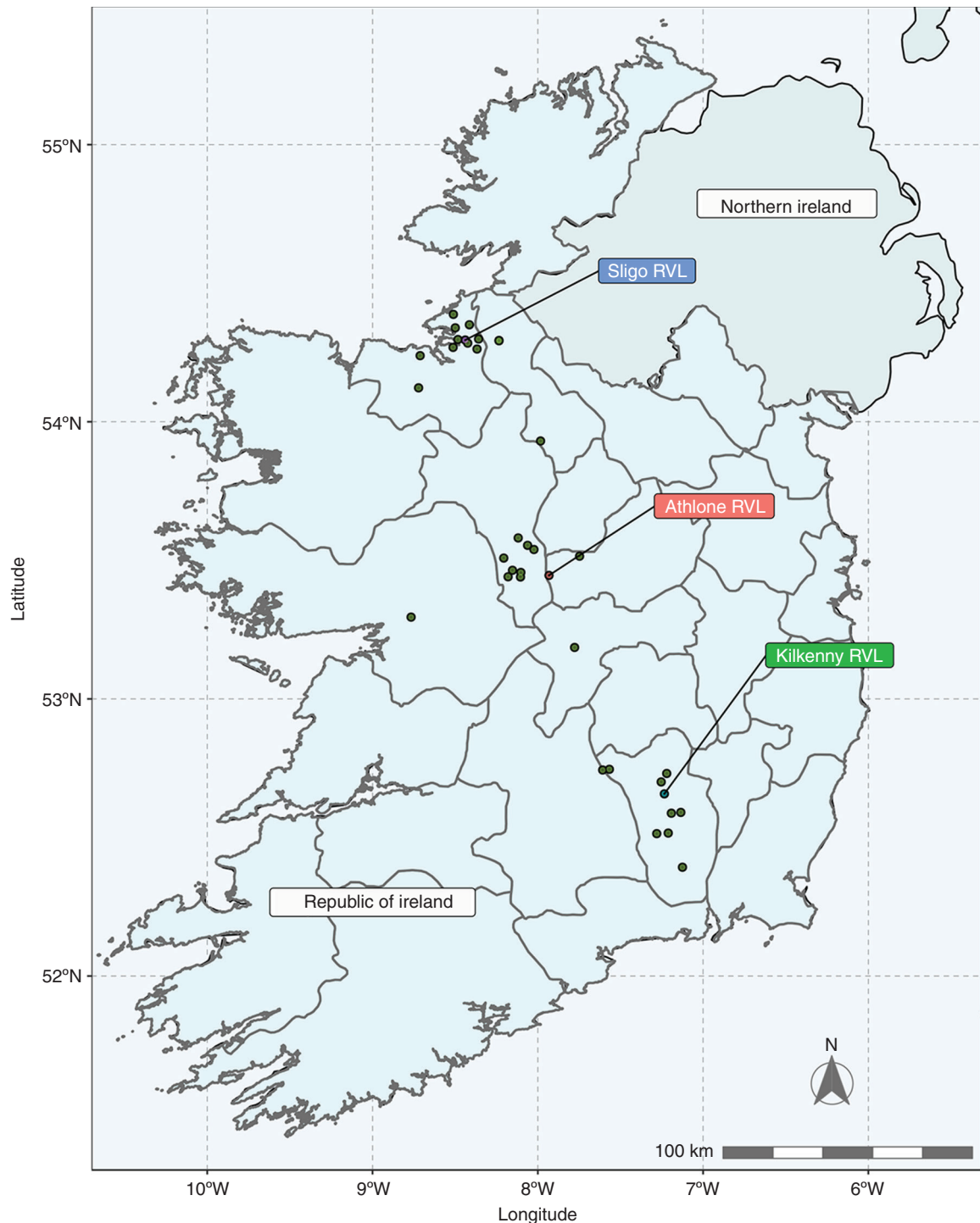


Figure 1 The location of the 33 participating flocks (green dots) and Athlone, Kilkenny and Sligo regional veterinary laboratories (RVLs).

Table 1 The seroprevalence of antibodies to recognised abortifacient agents at animal-level and flock-level as determined by blood sampling (n=30 flocks) in the first month of the study period and the prevalence of flock-level vaccination against these specific pathogens (n=33 flocks)

	Animal-level			Flock-level			Flock vaccination
	Positive	Total sampled	Positive (%)	Positive	Total sampled	Positive (%)	Number (proportion %)
Border disease antibody (ELISA)	2	172	1.2	2	14	14.3	0 (0.0)
Bovine virus diarrhoea antibody (ELISA)	0	122	0.0	0	9	0.0	0 (0.0)
<i>Chlamydophila abortus</i> (ELISA)	41	379	10.8	17	27	63.0	5 (15.2)
<i>Toxoplasma gondii</i> antibody (agglutination)	8	379	2.1	5	27	18.5	16 (48.3)
Schmallenberg virus antibody (ELISA)	12	379	3.2	5	27	18.5	0 (0.0)
Q fever antibody (ELISA)	4	366	1.1	3	30	10.0	0 (0.0)
<i>Salmonella</i> species OH titre	8	367	2.2	3	30	10.0	0 (0.0)

A seropositive flock was defined as a flock with one or more seropositive animals for the specific disease of interest.

Table 2 The number of total sheep submissions and age-specific sheep submissions received from the 33 participant flocks during 2016 expressed as a percentage of the total number of adult female sheep in the flock in January of that year

Flock number	Adult sheep submitted	Lambs submitted	Perinates submitted	Fetal submissions	Total submissions	Total adult female sheep in the flock in January 2016	Overall submission rate per adult female sheep in the flock in January 2016 (%)
1	9	2	1	0	12	180	6.7
2	1	2	3	6	12	130	9.2
3	4	6	10	10	30	200	15.0
4	3	2	7	8	20	200	10.0
5	7	10	9	0	26	210	12.4
6	3	2	1	0	6	160	3.8
7	1	5	2	1	9	100	9.0
8	8	20	38	2	68	190	35.8
9	16	52	31	14	113	418	27.0
10	4	24	30	7	65	340	19.1
11	5	2	1	5	13	70	18.6
12	4	5	7	3	19	200	9.5
13	19	25	6	10	60	686	8.7
14	12	5	25	0	42	200	21.0
15	2	2	10	0	14	150	9.3
16	1	1	0	1	3	100	3.0
17	18	12	3	22	55	400	13.8
18	7	12	11	11	41	360	11.4
19	15	50	17	30	112	810	13.8
20	8	7	3	17	35	140	25.0
21	5	13	11	23	52	250	20.8
22	6	3	16	0	25	145	17.2
23	3	0	0	0	3	120	2.5
24	8	14	7	0	29	190	15.3
25	7	5	7	3	22	190	11.6
26	13	12	10	32	67	210	31.9
27	5	4	3	8	20	195	10.3
28	8	17	4	16	45	170	26.5
29	16	28	23	17	84	440	19.1
30	3	2	0	2	7	180	3.9
31	21	12	35	3	71	360	19.7
32	6	7	10	5	28	240	11.7
33	11	7	15	6	39	185	21.1

Cause-specific mortality – fetuses (<day 130 of gestation)
Among 250 fetal submissions in which a diagnosis of abortion was made, *T gondii* (15.2 per cent) was the

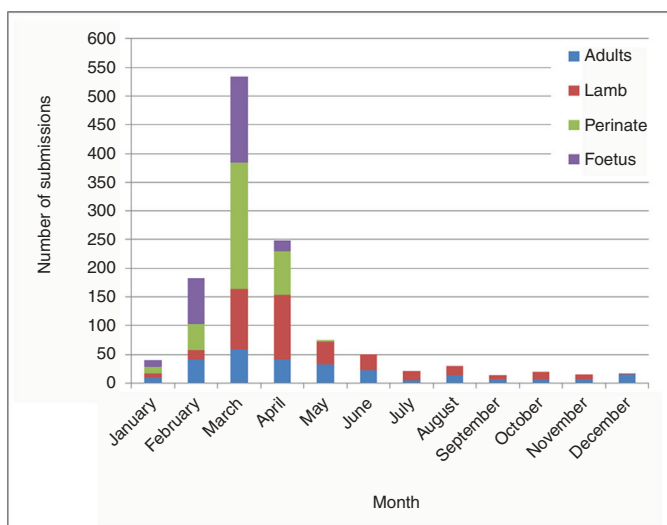


Figure 2 The temporal distribution of ovine adult, lamb, perinatal and fetal submissions from sentinel flocks to Athlone, Kilkenny and Sligo regional veterinary laboratories (RVLs) during the study period (n=1247 (259 adults, 370 lambs, 356 perinates, 262 fetuses)).

Table 3 The relative frequency of detection of abortifacient agents in fetal submissions from sentinel flocks to the regional veterinary laboratories (RVLs) during the study period (n=250)

Agent	Relative frequency (%)
No agent identified	66.8
<i>Toxoplasma gondii</i>	15.2
<i>Escherichia coli</i>	4.4
<i>Chlamydomonas abortus</i>	5.2
<i>Campylobacter</i> species	1.6
<i>Bacillus licheniformis</i>	1.2
<i>Streptococcus uberis</i>	1.2
<i>Trueperella pyogenes</i>	1.2
<i>Aeromonas hydrophila</i>	0.4
<i>Bibersteinia trehalosi</i>	0.4
<i>Listeria monocytogenes</i>	0.4
<i>Mannheimia haemolytica</i>	0.4
<i>Pseudomonas</i> species	0.4
<i>Staphylococcus aureus</i>	0.4
<i>Streptococcus</i> species	0.4
<i>Bacillus</i> species	0.4

pathogen identified with greatest frequency (table 3). Fetal membranes accompanied the fetus in 85 of these submissions.

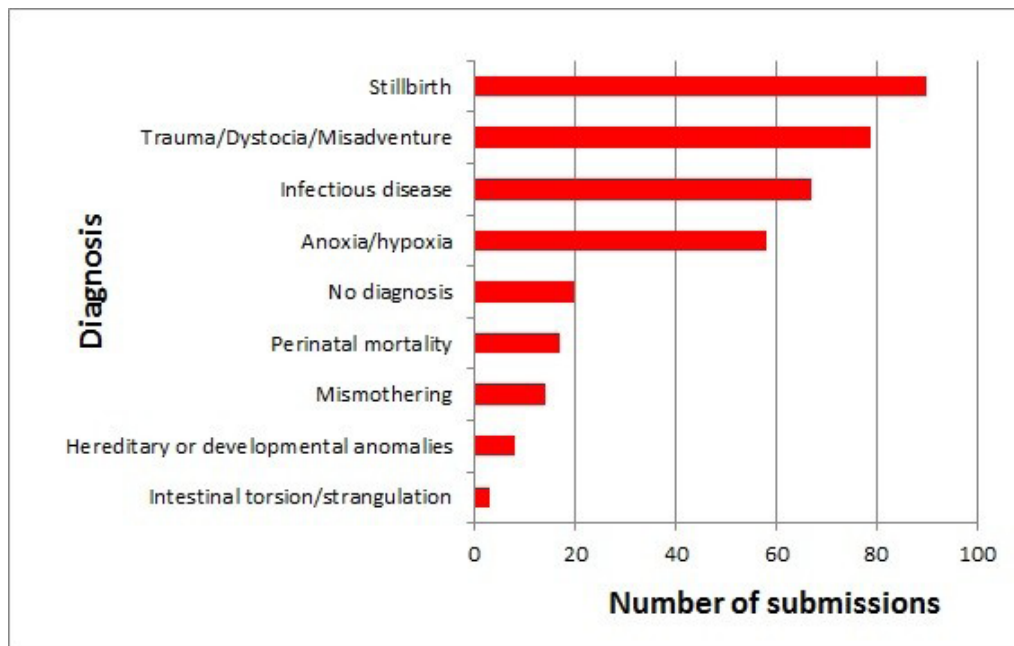


Figure 3 The frequency of gross postmortem diagnoses among ovine perinates (day 130 of gestation to 48 hours of age) in sentinel sheep flocks during the study period (n=356).

In addition, hereditary and developmental anomalies were diagnosed in three cases of fetal death comprising a single case each of ventricular septal defect, schistosomus reflexus and hydrups fetalis. Congenital pneumonia and placentitis were also diagnosed in a single case each; a pathogen was not detected in the former but the latter was associated with the detection of *C abortus* in the placenta.

Cause-specific mortality – perinates (day 130 of gestation to 48 hours of age)

Thirty four different diagnoses were recorded as causes of death among perinates submitted from the sentinel flocks to the RVLs during the study period. These could be broadly grouped into nine categories which are presented in figure 3. ‘Infectious diseases’ included enteritis, pneumonia, congenital toxoplasmosis, septic omphalitis/septic polyarthritis and septicaemia. ‘Hereditary and developmental anomalies’ included one case each of *atresia ilei*, *atresia recti*, anencephaly, hydrocephalus, renal dysplasia, congenital arthrogryposis, hypertrophic cardiomyopathy and defective development of the maxillary bone. PCR analyses for pestiviruses and SBV were negative in all eight lambs. ‘Perinatal mortality’ described those lambs which were viable but weak at birth and died shortly afterwards. Of 17 such cases, *C abortus* was identified in 3 while a significant pathogen was not identified among the remaining 14 cases.

Of 90 perinates in which a diagnosis of ‘Stillbirth’ was made, *C abortus* was identified in three; *T gondii* was identified in a single perinate. *Escherichia coli* (three lambs) and *Pasteurella* species (one lamb) were also identified in the fetal abomasal content. In most cases (82 lambs) a causative agent was not identified.

Cause-specific mortality – lamb (>2 days to <1 year of age) and adult sheep (≥1 year of age) carcasses

Among the 370 lamb carcasses and 259 adult sheep carcasses submitted from the sentinel flocks during the study period, 64 and 62 different causes of death were diagnosed, respectively. These diagnoses have been grouped into 13 separate categories as presented in figure 4.

‘Systemic disease’ included 75 cases (65 in lambs, 10 in adults) in which a diagnosis of septicaemia or bacteraemia was made. This category also included 14 cases of enterotoxaemia or toxoemia (7 in lambs, 7 in adults), 14 cases of peritonitis (4 in lambs, 10 in adults) and 13 cases of mastitis (all in adults). *Staphylococcus aureus* was isolated from eight of the mastitis cases while *M haemolytica* (n=3) and *Trueperella pyogenes* (n=1) were isolated less frequently. *E coli* was isolated with greatest frequency from cases of septicaemia or bacteraemia (n=38) in both lamb and adult sheep carcasses while *M haemolytica* (n=4) and *S aureus* (n=3) were detected with lesser frequency.

Individual alimentary tract disease diagnoses and respiratory disease diagnoses among lambs submitted from the sentinel flocks are presented in figures 5 and 6, respectively.

The agents associated with ‘Enteritis’ included *Eimeria* species (n=11; 10 lambs, 1 adult sheep), *Cryptosporidium parvum* (n=9; all lambs), *Salmonella typhimurium* (n=5; all adult sheep), *Salmonella* Dublin (n=1 lamb) and *Clostridium perfringens* type B (n=2 lambs).

From carcasses of both lambs and adult sheep in which a diagnosis of ‘Pneumonia’ was reached, *M haemolytica* (n=16) was isolated with greatest frequency with lower frequency of detection of *T pyogenes* (n=5) and *Pasteurella multocida* (n=2). Laryngeal chondritis was

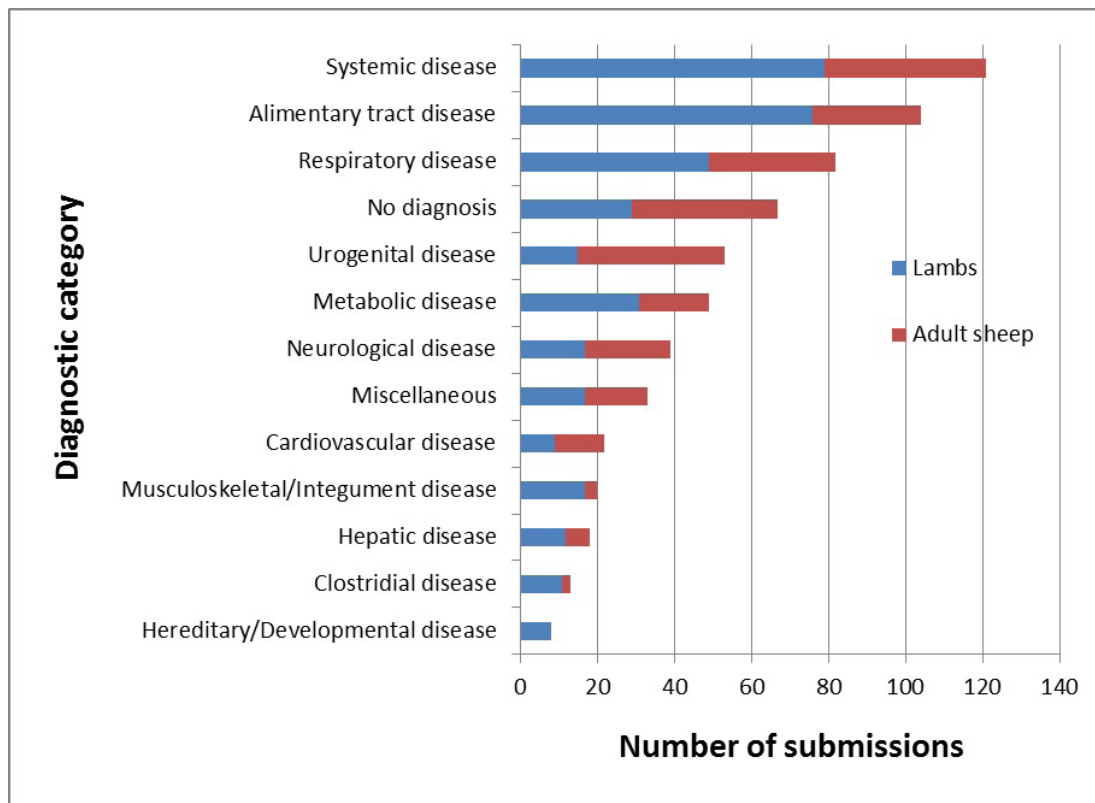


Figure 4 The frequency of categorised diagnoses in lamb carcasses (>2 days to <1 year of age; blue bars) and adult sheep carcasses (≥1 year of age; red bars) submitted from sentinel sheep flocks to Athlone, Kilkenny and Sligo regional veterinary laboratories (RVLs) during the study period (lambs: n=370; adult sheep: n=259).

diagnosed in four flocks and was evenly distributed between lambs (n=4) and adult sheep (n=6) carcasses. Eight of these cases were recorded in Texel sheep while the breed was not recorded in the other two cases.

The ‘No diagnosis’ category included cases in which a diagnosis was not reached following detailed examination and analysis (n=35; 22 lambs, 13 adults) as well as those where the carcass was unsuitable for examination due to predation or autolysis (n=32).

‘Urogenital disease’ included vaginal (n=15) or uterine (n=7) prolapse, metritis (n=9), nephrosis (n=9) and six cases of nephritis or pyelonephritis. All the cases of nephrosis were diagnosed in lambs less than five weeks of age. Six of the cases came from a single flock. In a single case, a toxic cause, oxalate, was identified.

‘Metabolic diseases’ included diagnoses of starvation or malnutrition (n=20 lambs), hypogammaglobulinaemia (n=7 lambs),

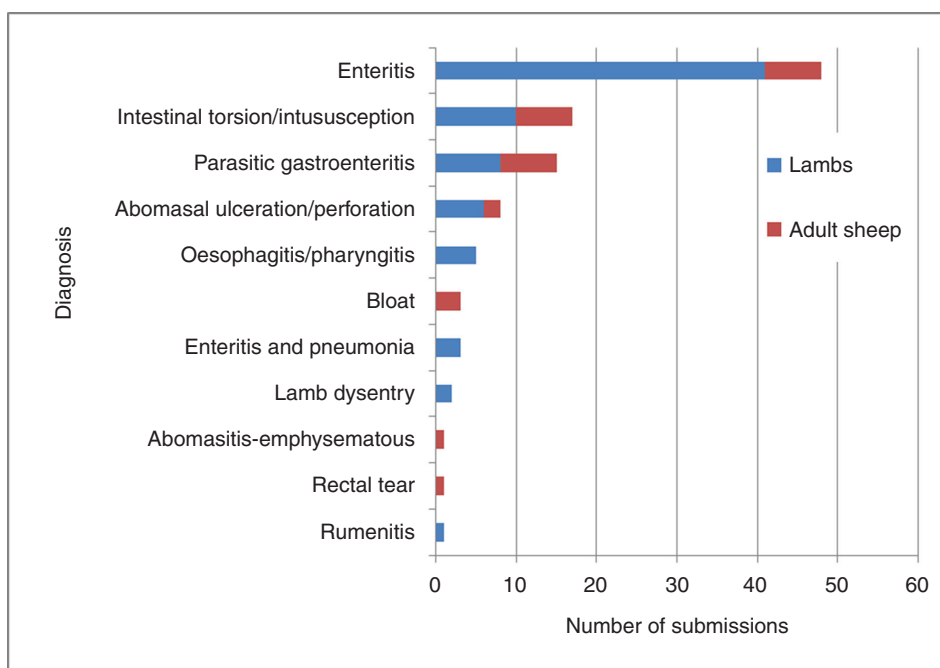


Figure 5 The frequency of individual alimentary tract disease diagnoses in lamb carcasses (>2 days to <1 year of age; blue bars) and adult sheep carcasses (≥1 year of age; red bars) submitted from sentinel sheep flocks to Athlone, Kilkenny and Sligo regional veterinary laboratories (RVLs) during the study period (lambs: n=76; adult sheep: n=28).

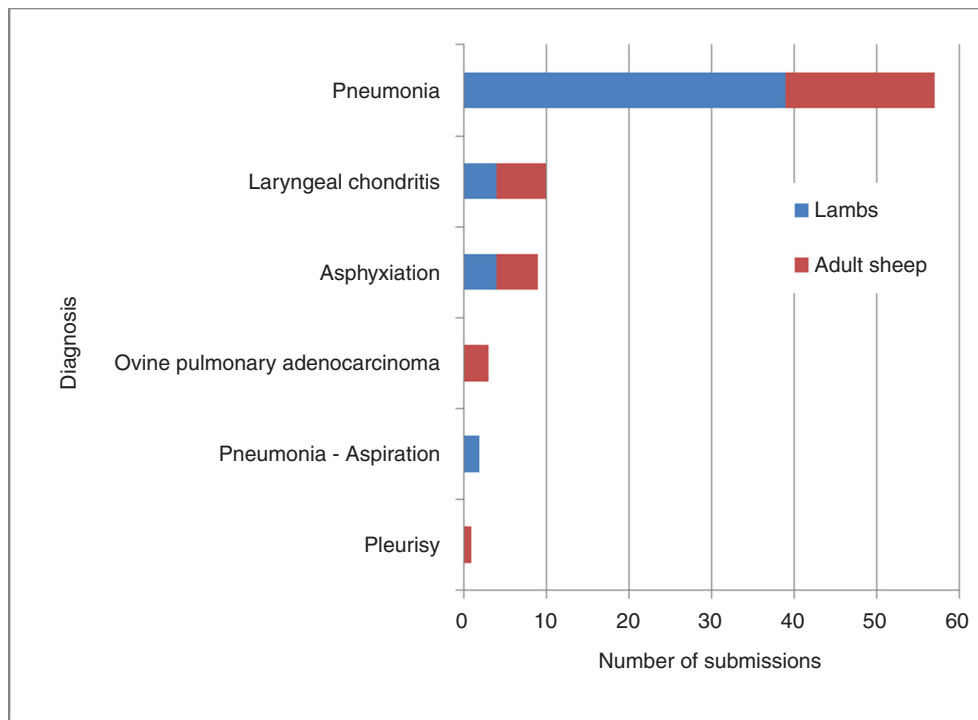


Figure 6 The frequency of individual respiratory disease diagnoses in lamb carcasses (>2 days to <1 year of age; blue bars) and adult sheep carcasses (≥ 1 year of age; red bars) submitted from sentinel sheep flocks to Athlone, Kilkenny and Sligo regional veterinary laboratories (RVLs) during the study period (lambs: $n=49$; adult sheep: $n=33$).

hypomagnesaemic tetany ($n=5$ adult sheep), ruminal acidosis ($n=4$; 3 lambs, 1 adult sheep), hypocalcaemia ($n=3$ adult sheep) and pregnancy toxoemia ($n=3$). Two diagnoses of copper poisoning in adult sheep in separate flocks were also included in this category.

Encephalitis ($n=23$; 6 lambs, 17 adult sheep) and cerebrocortical necrosis ($n=6$; 4 lambs, 2 adult sheep) were the neurological diseases most frequently recorded. *Listeria monocytogenes* (17 cases), louping ill virus (3 cases) and *Sarcocystis* species (1 case) were the agents detected among the cases of encephalitis.

'Miscellaneous' diagnoses included 27 cases of death due to trauma, tumours were identified in four adult sheep and electrocution was diagnosed as the cause of death in one lamb and one adult sheep which became entangled in an electric fence. The tumours identified included a single case each of mesothelioma of the peritoneal and pleural surfaces in a five-year-old ewe, an anaplastic metastatic sarcoma in the lungs and liver of a four-year-old ewe, an intestinal adenocarcinoma (signet ring type) in an ewe and an oligodendroglioma in a five-year-old ram.

Endocarditis ($n=7$; 3 lambs, 4 adult sheep), haemorrhage or shock ($n=7$) and pericarditis ($n=6$; 2 lambs, 4 adult sheep) were the cardiovascular disease diagnoses most frequently recorded. Cases of endocarditis were associated with the isolation of *T pyogenes* ($n=4$) and *Streptococcus suis* ($n=1$). *T pyogenes* was also identified in two cases of pericarditis (both adult sheep) while *M haemolytica* was identified in a single case in a lamb. Musculoskeletal and/or integument disease diagnoses were comprised primarily of septic

omphalitis or septic polyarthritis diagnoses ($n=9$ lambs), which were associated with *T pyogenes* ($n=3$) and *M haemolytica* ($n=1$), or abscessation ($n=4$).

Hepatic diseases included nine cases of hepatic abscessation, all in younger lambs, and two cases of acute fasciolosis in adult sheep. Cases of chronic fasciolosis were not recorded. Pulpy kidney disease ($n=11$ lambs) was the clostridial disease most frequently recorded.

Hereditary and/or developmental disease was the category of diagnoses recorded with the least frequency. *Atresia coli* was diagnosed in two lambs while *atresia jejuni*, *atresia ilei* and *atresia ani* were recorded in a single lamb each. Other diagnoses included patent ductus arteriosus and renal dysplasia, each recorded in a single lamb.

The 10 most frequently recorded individual diagnoses in lamb or adult sheep carcasses are presented in figure 7. Clustering of infectious disease was evident among some diagnoses, including those diagnoses which were most frequently recorded. Pneumonia was diagnosed in 24 flocks. In the two flocks in which it was most frequently recorded, five of a total of seven cases and three of a total of six cases, respectively, were submitted for examination on the same day. A similar trend was observed among enteritis cases (eight cases in one flock, five of which were submitted within a three-day period) and even among non-infectious disease cases such as trauma (eight from a single flock, six of which were submitted on the same day).

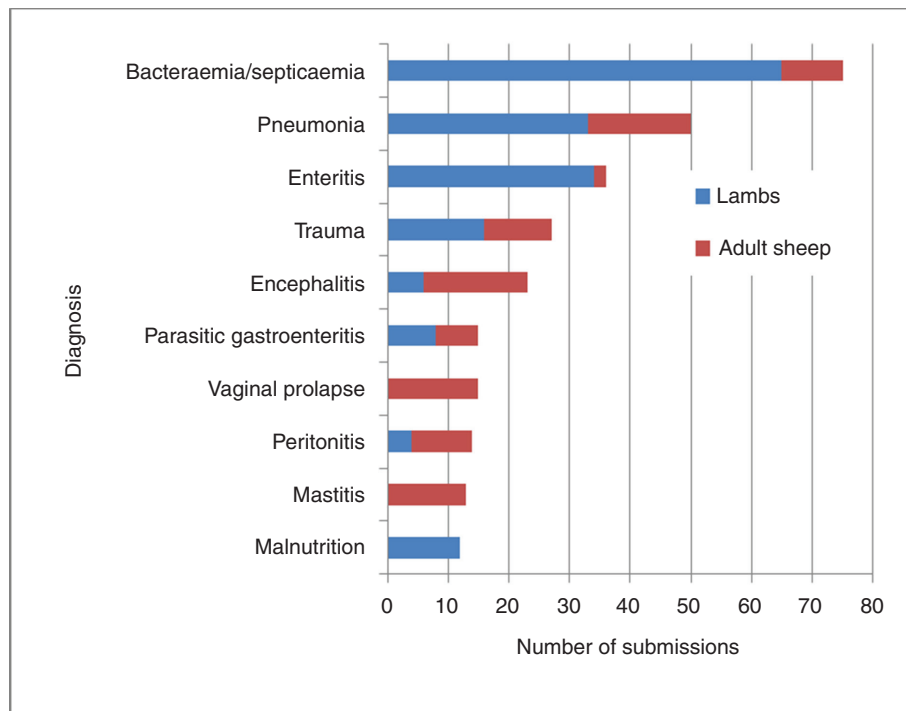


Figure 7 The 10 most frequently recorded individual diagnoses in lamb (>2 days to <1 year of age) and adult sheep (≥1 year of age) submitted from sentinel sheep flocks to Athlone, Kilkenny and Sligo regional veterinary laboratories (RVLs) during the study period.

Detection of specific pathogens in sheep from vaccinated flocks

Fifteen flocks that reported vaccinating against clostridial disease recorded a clostridial disease diagnosis during the study period. These diagnoses were confined to lambs or perinates in 12 of these 15 flocks. Among the 26 clostridial disease diagnoses, *Clostridium perfringens* type D was the agent identified with greatest frequency (n=13) with *C perfringens* type B isolated in a further three cases. *Clostridium sordellii* was isolated from two cases of malignant oedema (one lamb and one adult sheep) from the same flock. Of the 23 flocks engaged in *M haemolytica* and *B trehalosi* vaccination, *B trehalosi* was identified in a single fetus and *M haemolytica* was recorded in seven respiratory disease cases (four lambs, three adult sheep) submitted from six different flocks.

T gondii was detected in 31 fetuses (from 10 flocks) among the 384 submissions (203 fetuses, 181 perinates) submitted from the 16 flocks which reported vaccination of their flock against *T gondii* infection. *C abortus* was detected in nine fetuses and five perinates submitted from four of the five flocks which engaged in vaccination against EAEs.

Discussion

This is the first published study of laboratory-diagnosed mortality and cause-specific mortality in Irish lowland sheep flocks. The findings are an important addition to knowledge of current disease trends and in determining the current baseline levels of endemic disease in the national sheep flock. In the absence of robust, reliable data on the frequency of occurrence of different diseases in different age categories of sheep,

it has not been possible to accurately assess the impact of different disease control or prevention management interventions in Irish sheep flocks.

Hoinville *et al*¹⁷ defined sentinel surveillance as the repeated collection of data or samples from selected sites which act as proxies for the entire population. It represents a relatively underexploited resource in animal disease surveillance both in Ireland, and internationally.¹⁸ When compared with passive surveillance, McCluskey¹⁹ argued that, by focusing on specific subpopulations, it represents a more cost-effective approach. Both passive and sentinel surveillance can facilitate the identification of new and emerging diseases and pathogens. However, unlike passive surveillance, which is influenced by the factors which determine submission behaviour to veterinary diagnostic laboratories,^{5, 6} sentinel surveillance can highlight changes in the prevalence or incidence of a pathogen or disease over time as well as allowing assessment of the efficacy of potential disease control interventions.¹⁹ Some of the diseases reported in this study, such as trauma, tumours, mastitis and hereditary or developmental anomalies may be evident to the farmer (or occur at such a low frequency) that they will only sporadically appear in voluntary submissions to laboratories²⁰ which renders any assessment of their prevalence in the Irish national sheep flock, based on passive surveillance, unreliable. The choice of sentinel flocks is also important; the flocks enrolled and repeatedly sampled in this study constituted a convenience sample of the target population. Halliday *et al*²¹ discussed the associations between the sentinel and target populations and the need for a spatial association (but not necessarily overlap) between

both. In this study, while the spatial distribution of the sentinel flocks was clustered close to the RVL sites in the north-west, midlands and south-east, the sentinels which were chosen from sheep-dense regions of the country represent a specific subset of the target population, which ensures a very close relationship between the two populations.

Studies of ovine mortality in the literature typically differ in terms of methodology, terminology or the rate measures employed which render any comparison of findings between these studies problematic. Methodological approaches have variously relied on veterinary student-administered²² or farmer-administered questionnaires,²³ farmer-performed neonatal lamb postmortem examinations²⁴ or a combination of veterinary or flock-manager diagnostic postmortem procedures²⁵ to determine mortality or cause-specific mortality rates. Terminology such as 'neonatal' or 'perinatal' also vary between studies²⁴⁻²⁸ as have rate measures such as 'mortality between scanning and sale'²⁹ and 'mortality between 24 hours and weaning'.²⁵

Identification of a suitable rate measure in such studies is challenging as the denominator is constantly changing during the lambing season. Statistical methods for accommodating the change of denominator across the lambing season and obtaining true mortality rates (eg, mortality per time at risk) requires detailed data recording at an individual animal level which was not available. Douglas and Sargison²² recently reported a mean perinatal mortality rate of 10 per cent although neither the definition of 'perinatal' nor the denominator used was clarified. Green *et al*²³ reported 9 per cent–11 per cent mortality between days 0 and 7 after birth while Binns *et al*³⁰ reported losses of between 6 per cent and 12.5 per cent between scanning and 48 hours of age. Mellor and Stafford²⁹ have recorded overall lamb mortality between scanning and sale ranging from 10 per cent to 25 per cent. In the present study, using the number of adult female sheep in the flock in January as the denominator, the authors identified a median overall submission rate (as a proxy for mortality rate) from the sentinel lowland sheep flocks to the RVL, of 13.8 per cent and median fetal and perinatal submission rates of 2.1 per cent and 3.5 per cent, respectively.

Comparisons of the relative frequency of specific disease categories identified in this study through sentinel surveillance with national sheep surveillance data³¹ from primarily passive surveillance activities highlights how a sentinel approach can usefully supplement passive surveillance. Among lambs and adult sheep, considered together, systemic diseases (eg, bacteraemia or septicaemia) were the most common category of diagnoses recorded while clostridial diseases were diagnosed relatively infrequently by sentinel surveillance when compared with passive surveillance. The frequency of detection of respiratory diseases

and metabolic diseases was almost the same by both methods as was the proportion of carcasses in which no diagnosis was made (approximately 10 per cent). It is likely that differences in detection of some categories of disease, either through clinical presentation challenges, or the willingness to submit, reflect the drivers of submission behaviour in passive surveillance systems where sudden death (as is typically the result of clostridial infections) is more likely to prompt veterinary diagnostic submissions.

The findings recorded in fetal and perinatal lamb submissions are also of note, specifically the relatively high proportion of cases in which a causative pathogen was not identified (67.9 per cent and 83.1 per cent, respectively). Nonetheless, the diagnostic rate in the present study was broadly within international norms.³²⁻³³ The reasons for failure to detect causative pathogens in fetuses and stillbirths are many and varied: non-infectious causes of abortion or stillbirth, poor carcase preservation which can limit diagnostic capabilities, delays between fetal death and expulsion, or the sensitivity of existing test methods to list but a few. Of cases in which abortion was diagnosed, it was disappointing that only one-third of these fetal submissions were accompanied by fetal membranes for examination. It is quite likely, considering the high rate of *C abortus* exposure demonstrated by flock serology, that the low fetal membranes submission rate also negatively impacted the diagnostic rate for some abortifacients, in particular *C abortus*, in the present study population despite the availability of PCR analysis.

The authors recorded median submission rates for lambs and adults of 3.0 per cent and 2.8 per cent respectively. These rates compare favourably with previous studies from Australia (mean postweaning mortality of 4.6 per cent³⁴), New Zealand (4.9 per cent among sheep aged four months of age and older³⁵) and Canada (total lamb mortality of 12.1 per cent³⁶). The relative frequency of diagnosed causes of death in these age categories in the present study differed somewhat from those studies referenced where pregnancy toxemia³⁵ and starvation³⁶ were identified as the most frequently recorded cause of death in lambs. Nash *et al*²⁵ reported respiratory disease as the most frequently (18 per cent) recorded diagnosed cause of death in preweaned lambs. In the present study, while respiratory disease in lambs represented a significant (13.2 per cent) cause of death, it was less frequently diagnosed than systemic disease (21.4 per cent) or alimentary tract disease (20.5 per cent). Among respiratory disease diagnoses, detections of laryngeal chondritis (n=10; adult sheep and lambs combined) and ovine pulmonary adenomatosis (OPA; n=3 adults) were relatively infrequent but interesting findings. Laryngeal chondritis is over-represented in the Texel breed but its exact cause is the subject of much ongoing

debate.³⁷ Anecdotal reports in Ireland have suggested that it is a significant disease in a small number of flocks but prevalence studies are still awaited. Detection of the condition in four separate flocks in this present study supports a need for further research in this area in Ireland. Lovatt and Strugnell,²⁰ in a study of 106 ewes examined in a fallen stock centre in the UK, reported mastitis as the most frequently recorded (10.4 per cent) diagnosed cause of death; all cases were recorded between April and July. The authors similarly recorded a high incidence of mastitis with a similar seasonal distribution in ewes (13 cases, 10 of which were recorded between March and May). That study²⁰ also reported OPA incidence of 6 per cent in ewes. Recently, Lee *et al*³⁸ reported 10 cases of OPA from five different flocks in an abattoir survey of 1911 adult lungs in Ireland. The findings of the present study of three OPA cases from three different flocks agree with the findings of that study that this is a relatively rare disease but is present in sheep in Ireland.

Diseases of the alimentary tract in adult sheep and lambs were the second most frequently diagnosed cause of death (16.5 per cent) in these age categories (when combined). This was due primarily to the prevalence of enteritis in lambs (11.9 per cent) while parasitic gastroenteritis was also frequently recorded in both age categories. Urogenital disease (14.7 per cent) was more frequently diagnosed than alimentary tract disease (10.8 per cent) among adult sheep, however, reflecting the significance of diagnoses such as vaginal or uterine prolapse (8.5 per cent) and metritis (3.5 per cent) in this age category. Among neurological disease diagnoses, the consistent association of *L monocytogenes* with diagnoses of encephalitis (present in all 17 adult cases), all of which succumbed to the disease between February and May, underlines the potential importance of contaminated forage in the epidemiology of this disease.

Vaccination is frequently and widely recommended as a disease control measure in sheep flocks. Only one of the participant flocks in this study did not engage in vaccination against any disease. Among those that did engage in vaccination, the pathogens against which vaccination was undertaken were identified in submissions from those flocks. This finding is not wholly unexpected as vaccination of the flock does not infer 'sterile immunity' nor does detection of a pathogen on postmortem examination necessarily infer causation. Without data on vaccine storage, administration frequency or checks to validate that all submitted animals actually received the vaccine, it is impossible to be more specific about the reasons for these findings; however, it can be speculated that, at least some of the clostridial disease in lambs may be due to insufficient maternal transfer of immunity to the newborn lamb or waning passive immunity in older lambs. Uzal and Songer³⁹ reported that, although

widely used, there can be variations in the individual response or manufacturer quality at play in determining the response of sheep flocks to multivalent clostridial vaccination. The relatively infrequent vaccination of flocks against EAEs was also an interesting finding. In this study, the detection rate of *C abortus*-associated abortion was relatively low (5.2 per cent) among all fetuses submitted to the RVLs; however over half of these detections were in fetal submissions from the five flocks engaging in vaccination against EAE. Successive reports from passive surveillance of ovine abortion in Ireland^{3 31} have consistently detected *C abortus* in 20–26 per cent of ovine fetal submissions to the RVLs, ranking as the second most frequently diagnosed infectious cause of abortion in sheep in Ireland. These reports, one might expect, would prompt higher EAE vaccination rates among flocks in the study population. It is possible however, that the knowledge that occasional EAE outbreaks can still occur in vaccinated flocks or reports on the detection of the vaccinal strain of *C abortus* in some outbreaks in recent years⁴⁰ had an effect on farmers' willingness to vaccinate against this disease.

The authors readily acknowledge that it is possible that full ascertainment of cases may have suffered from some farmers failing to submit all ovine mortality from their flock during the study period or by being unaware of early term abortions in ewes. Unfortunately, data on scanning rates were not available from the study flocks. It has previously been noted that few farmers in Ireland record the number of lambs born per ewe or scan pregnant ewes.⁴¹ The lack of this data can affect the accuracy of detection of some specific diseases, particularly the prevalence of abortifacient agents. Nevertheless, considering the collection service provided and waiving of all fees associated with participation in the study, it is likely that the authors have achieved high case ascertainment and that their data give an accurate reflection of mortality in these flocks.

Holmoy *et al*⁴² suggested that the relative frequency of certain diagnosed causes of perinatal death (eg, dystocia or hypogammaglobulinaemia) in flocks which lamb indoors may differ from those which lamb outdoors due to differences in the intensity of lambing surveillance. Data on whether sheep were due to lamb indoors or outdoors were unavailable in this study and it is possible, therefore, that the overall number of submissions, and those of perinates specifically, may have been influenced by the lambing system on farm. Candidate flocks of the present study were also larger than the national average flock size, selected based on a willingness to participate, managed by flock owners engaged in discussion groups (and therefore possibly more motivated than average) and, with the exception of one flock, entirely lowland flocks. While these potential biases suggest that the findings of this study may not be immediately generalisable to the broader

Irish national flock, they certainly provide a more accurate understanding of mortality and cause-specific mortality in lowland flocks than that achievable by passive surveillance alone. Future sentinel surveillance initiatives in Ireland should address greater representativeness among candidate flocks and should seek to ensure ewes are scanned and all lambs are identified and recorded at birth.

In conclusion, the findings of the present study reflect the laboratory submission rate (a proxy for mortality rate) in sentinel Irish lowland sheep flocks and provide a more accurate measure of the frequency of recognised causes of ovine abortion and mortality among different age groups in these flocks. The findings of the present study also represent the first published report of veterinary diagnostic laboratory sentinel surveillance in Ireland. The use of sentinel flocks to complement existing passive animal disease surveillance activities would represent an enhanced surveillance system in Ireland for endemic, novel or exotic disease and would likely prove to be particularly useful in monitoring baseline trends over time.

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References

- 1 Keady T, Hanrahan K. The Sheep Industry – its recent evolution. 2016 <https://www.teagasc.ie/media/website/publications/2016/The-Sheep-Industry-its-recent-evolution-2-Aug-2016.pdf> (accessed 18 Sep 2018).
- 2 DAFM. The 2017 Department of Agriculture, Food and Marine Sheep and Goat census. 2018. <https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/animalidentificationandmovement/NationalSheepAndGoatCensus2017230718.pdf> (Accessed 18 Sep 2018).
- 3 DAFM. Department of Agriculture, Food and Marine. The 2016 DAFM/AFBI All-island animal disease surveillance report. 2017 <https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/labservice/rvlreports/AIDSRReport016230118.pdf> (accessed 18 Sep 2018).
- 4 DAFM. The Regional Veterinary Laboratories: Current and Future Roles. In: Irish Management Institute project presented to the Chief Veterinary Officer: Department of Agriculture, Food and Marine, 2007. Unpublished. 2007.
- 5 Robinson PA, Epperson WB, Huston CL, et al. Factors influencing diagnostic sample submission by food animal veterinarians in Mississippi. *Vet Ital* 2012;48:31–9.
- 6 Sawford K, Vollman AR, Stephen C. A focused ethnographic study of Alberta cattle veterinarians' decision making about diagnostic laboratory submissions and perceptions of surveillance programs. *PLoS One* 2013;8:e64811.
- 7 Doherr MG, Audigé L. Monitoring and surveillance for rare health-related events: a review from the veterinary perspective. *Philos Trans R Soc Lond B Biol Sci* 2001;356:1097–106.
- 8 Gauci C, Melillo Fenech T, Gilles H, et al. Sentinel Surveillance: an option for surveillance of infectious intestinal disease. *Eurosurveillance* 2007;12:13–14.

- 9 Teutsch SM, Churchill RE. Principles and practice of public health surveillance. Oxford University Press: Oxford, 2000.
- 10 Barkallah M, Gharbi Y, Hassena AB, et al. Survey of infectious etiologies of bovine abortion during mid- to late gestation in dairy herds. *PLoS One* 2014;9:e91549.
- 11 Saad NM, Hussein AAA, Ewida RM. Occurrence of *Toxoplasma gondii* in raw goat, sheep, and camel milk in Upper Egypt. *Vet World* 2018;11:1262–5.
- 12 O'Neill R, Mooney J, Connaghan E, et al. Patterns of detection of respiratory viruses in nasal swabs from calves in Ireland: a retrospective study. *Vet Rec* 2014;175:351–6.
- 13 Barrett D, More SJ, O'Neill R, et al. Prevalence and distribution of exposure to Schmallenberg virus in Irish cattle during October 2012 to November 2013. *BMC Vet Res* 2015;11:267–76.
- 14 Taylor MA, Coop RL, Wall RL. Veterinary Parasitology. 4th edn: Wiley-Blackwell, 2015.
- 15 Duignan G, Fagan J, Joyce C, et al. Diagnosing acute larval paramphistomosis in ruminants. *Vet Rec* 2017;180:618.
- 16 Henriksen SA, Aagaard K. A simple flotation and McMaster method. *Nord Vet Med* 1976;28:392–7.
- 17 Hoinville LJ, Alban L, Drewe JA, et al. Proposed terms and concepts for describing and evaluating animal-health surveillance systems. *Prev Vet Med* 2013;112:1–12.
- 18 Rabinowitz PM, Gordon Z, Holmes R, et al. Animals as sentinels of human environmental health hazards: An evidence-based analysis. *Ecohealth* 2005;2:26–37.
- 19 McCluskey BJ. Use of sentinel herds in monitoring and surveillance systems. In: Salman MD, ed. Animal disease surveillance and survey systems: methods applications: Iowa: Iowa State Press, 2003;119–33.
- 20 Lovatt FM, Strugnell BW. An observational study involving ewe postmortem examination at a fallen stock collection centre to inform flock health interventions. *Vet Rec* 2013;172:504.2–504.
- 21 Halliday JE, Meredith AL, Knobel DL, et al. A framework for evaluating animals as sentinels for infectious disease surveillance. *J R Soc Interface* 2007;4:973–84.
- 22 Douglas F, Sargison ND. Husbandry procedures at the point of lambing with reference to perinatal lamb mortality. *Vet Rec* 2018;182:52–4.
- 23 Green L, Cox I, Rizvi S, et al. The prevalence and risk factors for mortality in spring lambing flock: a cross sectional study. Proceedings of the Spring Meeting of the Sheep Veterinary Society. Scarborough, Yorkshire, 1998;22:51–5.
- 24 Gascoigne E, Bazeley K, Lovatt F. Can farmers reliably perform neonatal lamb post mortems and what are the perceived obstacles to influencing lamb mortality? *Small Ruminant Research* 2017;151:36–44.
- 25 Nash ML, Hungerford LL, Nash TG, et al. Risk factors for perinatal and postnatal mortality in lambs. *Vet Rec* 1996;139:64–7.
- 26 Fragkou IA, Mavrogianni VS, Fthenakis GC. Diagnostic investigation of cases of deaths of newborn lambs. *Small Ruminant Research* 2010;92:41–4.
- 27 Holmøy IH, Waage S. Time trends and epidemiological patterns of perinatal lamb mortality in Norway. *Acta Vet Scand* 2015;57:65–76.
- 28 Refshauge G, Brien FD, Hinch GN, et al. Neonatal lamb mortality: factors associated with the death of Australian lambs. *Anim Prod Sci* 2016;56:726–35.
- 29 Mellor DJ, Stafford KJ. Animal welfare implications of neonatal mortality and morbidity in farm animals. *Vet J* 2004;168:118–33.
- 30 Binns SH, Cox IJ, Rizvi S, et al. Risk factors for lamb mortality on UK sheep farms. *Prev Vet Med* 2002;52:287–303.
- 31 DAFM. Department of Agriculture, Food and Marine. The 2015 DAFM/AFBI All-island animal disease surveillance report, 2016. <https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/labservice/rvlreports/AIDSRReport016230118.pdf> (accessed 9 Oct 18).
- 32 Kirkbride CA, in D. ovine abortions and stillbirths. *J Vet Diag Invest* 1784;1993:398–402.
- 33 Oporto B, Barandika JF, Hurtado A, et al. Incidence of ovine abortion by *Coxiella burnetii* in northern Spain. *Ann N Y Acad Sci* 2006;1078:498–501.
- 34 Campbell AJD, Broekhuizen A, Curtis K, et al. A survey of post-weaning mortality of sheep in Australia and its association with farm and management factors. *Anim Prod Sci* 2014;54:783–90.
- 35 Davis GB. A sheep mortality survey. Proceedings of the 2nd International Symposium on Veterinary Epidemiology and Economics, 1979:106–10. <http://www.sciquest.org.nz>. (Accessed 10 Oct 2018).
- 36 Dohoo IR, Curtis RA, Finley GG. A survey of sheep diseases in Canada. *Can J Comp Med* 1985;49:239–47.
- 37 Sigurðardóttir ÓG, Jörundsson E, Friðriksdóttir V. Laryngeal Chondritis in Sheep in Iceland. *J Comp Pathol* 2016;155:310–3.
- 38 Lee AM, Wolfe A, Cassidy JP, et al. First confirmation by PCR of Jaagsiekte sheep retrovirus in Ireland and prevalence of ovine pulmonary adenocarcinoma in adult sheep at slaughter. *Ir Vet J* 2017;70:33–45.
- 39 Uzal FA, Songer JG. Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. *J Vet Diagn Invest* 2008;20:253–65.
- 40 Wheelhouse N, Aitchison K, Laroucau K, et al. Evidence of *Chlamydia abortus* vaccine strain 1B as a possible cause of ovine enzootic abortion. *Vaccine* 2010;28:5657–63.
- 41 Barrett D, O'Neill R, Sammin D, et al. The impact of infection with Schmallenberg virus on weaning rate in Irish sheep flocks. *Prev Vet Med* 2015;122:332–8.
- 42 Holmøy IH, Waage S, Granquist EG, et al. Early neonatal lamb mortality: postmortem findings. *Animal* 2017;11:295–305.

