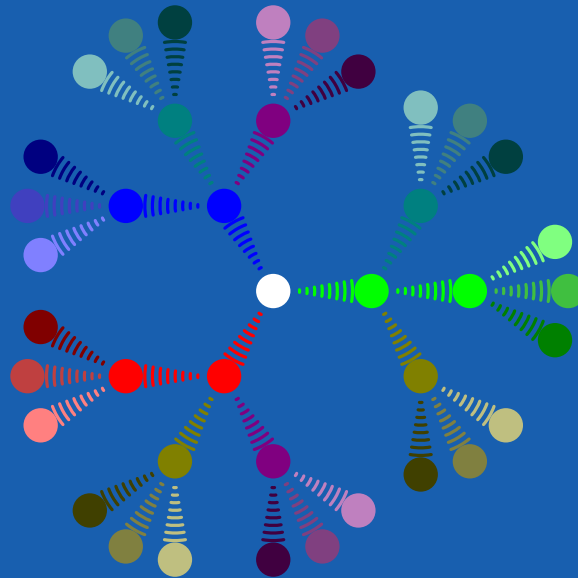**An Roinn Talmhaíochta, Bia agus Mara**
Department of Agriculture, Food and the Marine

All-Island Animal Disease Surveillance Report
Veterinary Laboratory Service

2018

Dept. of Agriculture, Food and the Marine
14 October, 2019

Acknowledgements

Table of Contents

Introduction

Animal Disease Surveillance Report

Veterinary Laboratory Service

October 3, 2019

In an animal health context, surveillance broadly refers to the collection, processing and timely dissemination of animal health information. The Department of Agriculture, Food and the Marine (DAFM) has a central role in animal health surveillance in Ireland. Surveillance is carried out for many reasons, and the information collected comes in many forms.

For a relatively small number of diseases, the surveillance is initiated centrally by DAFM or other bodies such as Animal Health Ireland. This surveillance is known as active surveillance, and it is typically achieved through coordinated testing programmes. The reasons for active surveillance include control of high-impact diseases such as bovine spongiform encephalopathy (BSE), disease eradication as in the case of bovine tuberculosis, bovine brucellosis and BVD, and proof of freedom from diseases which could potentially affect trade, such as enzootic bovine leukosis and bluetongue.

Passive surveillance

Passive surveillance refers to surveillance initiated by the observers of animals. It encompasses both the reporting of notifiable diseases to the authorities and the voluntary submission of carcases and samples to veterinary laboratories. Passive surveillance has been, and continues to be, Ireland's mainstay in detecting animal diseases. A key role of the laboratories operated by DAFM and others is to provide those working with animals, such as livestock owners and veterinary practitioners with the diagnostic support required to inform their work. While this supporting role can be invaluable to the farmers and vets availing of it, veterinary laboratories are also a critical part of the national infrastructure required for early detection of new/emerging or exotic diseases (i.e. diseases which are not currently present in Ireland). Although the probability of incursion of an exotic disease at any given time may be very low, the consequences of such an event are potentially very significant. Therefore, although less visible to stakeholders, the role of the laboratories in vigilance for exotic diseases is arguably even more important to the wider agri-food industry in safeguarding animal health and export trade.

Climate change

The long hot summer of 2018 appears to have had a positive effect on the overall health of livestock. It also posed challenges for many farmers in the south and east of the country - in ensuring that livestock had continued access to drinking water and both sufficient summer pasture and enough winter fodder with such an extended period of poor grass growth. While the attribution of an increasing number of unusual weather events like long hot summers and severe storms to climate change is a matter of debate, there is now a broad societal acceptance of the concept of climate change induced by human activity, and of the urgent need for climate action, with agriculture receiving particular attention in respect of both mitigation and adaptation. Improving the efficiency of livestock production as a possible means to reduce the carbon footprint per kilogram of meat or milk produced is one key aspect of mitigating the impact of farming. Therefore laboratory diagnostic support is likely to be needed more than ever to provide the information that farmers and their vets will need to prevent and control diseases, thereby minimising morbidity and mortality and maximising productivity. Climate change may also play a more direct role in increasing risks to animal health if it allows for an expansion in the geographic range, abundance and/or activity of arthropod vectors of livestock diseases which heretofore have been exotic to Ireland - yet another reason for robust surveillance.

The report

It is in this context that we present the 2018 Animal Disease Surveillance Report. While it conveys something of the scale and range of laboratory activities undertaken every year, this report cannot adequately reflect the effort and dedication of those *behind the scenes* in the provision of laboratory diagnostic services, nor the challenges inherent in extracting meaningful surveillance information from a voluntary diagnostic caseload. We must also acknowledge the critical role played by our clients (farmers and vets) who, by making the effort to avail of laboratory services, are not only taking full responsibility for the health and welfare of the animals under their own care, but are also indirectly contributing to the store of knowledge available to the wider community in maintaining the favourable health and welfare status of the national herd. Finally, a particular word of thanks to Cosme Sánchez-Miguel, whose tireless efforts have ensured the timely production of this report.

We are pleased to commend this report to our readers. For further information on animal health surveillance, please also visit our Animal Health Surveillance webpage.

Donal Sammin

Mícheál Casey

Diseases of Cattle

2018

Overview of Cattle Diseases

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The Regional Veterinary Laboratories (RVLs) of the Department of Agriculture, Food and the Marine (DAFM) are engaged primarily in scanning (passive) surveillance by gathering data from *post-mortem* and clinical sample submissions. Analysis of this data provides an insight into trends of disease incidence and causes of mortality on Irish farms, thereby informing decision-making relevant to disease control at a national level. Tables and charts are generated with test results and *post-mortem* diagnoses from voluntary submissions of material (carcasses and clinical samples) to RVLs by farmers through their private veterinary practitioners (PVPs). Therefore, it should be noted that data reflects only those cases where PVPs considered it appropriate to request laboratory investigation and the herdowner was motivated to deliver the carcass to an RVL.

Diseases of Cattle

This section presents the most commonly diagnosed causes of death in cattle presented for post-mortem examination at RVLs.

The range of diagnoses of animals submitted for *post-mortem* examination varies according to age of the animal, thus the results in this section are presented by age category. In order to facilitate presentation and comparison, conditions which affect given systems have been grouped together.

During 2018, 2902 cattle carcasses were submitted for examination. Geographical distribution of herds submitting bovine cases, colour coded by RVL where the carcases were examined, is illustrated in Figures 1.

Neonatal Calves (birth to one month of age)

The trend of gastrointestinal infections being the most frequently diagnosed cause of death in neonatal calves continued in 2018 (Table 1 & Figure 2). The deaths of almost thirty *per cent* of calves in this age group were attributed to gastrointestinal infections. Not surprisingly, a number of these cases had hypogammaglobulinaemia recorded as well, indicating failure of passive transfer of humoral immunity from dam to calf.

Systemic infections (sepsis) continued as the second most frequently diagnosed cause of death in DAFM laboratories. In the last three years, systemic infections reached a peak of 24 *per cent* of deaths in 2016 (DAFM, 2016), but in 2018 the percentage of neonatal calves with systemic infections fell to 17.8 *per cent*.

● Athlone RVL	● Cork RVL	● 5	● 10
● Dublin RVL	● Kilkenny RVL		
● Limerick RVL	● Sligo RVL		

Figure 1: Distribution of bovine carcasses. Bovine carcasses (excluding foetuses), aggregated and mapped at their DED (District Electoral Division) and colour-coded by the Regional Veterinary Laboratory of submission (n= 2902)

Table 1: Conditions most frequently diagnosed on *post-mortem* examinations of bovine neonatal calves in 2018 (n= 845).

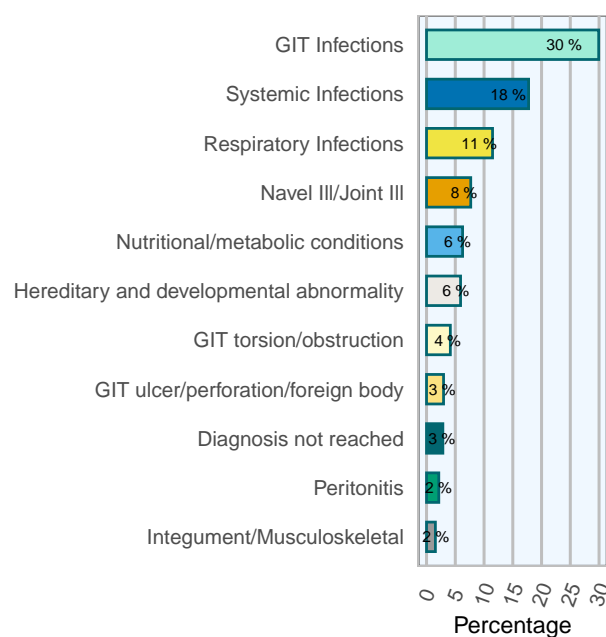| Category	No. of Cases	Percentage
GIT Infections	253	29.9
Systemic Infections	150	17.8
Respiratory Infections	97	11.5
Navel Ill/Joint Ill	65	7.7
Nutritional/metabolic conditions	53	6.3
Hereditary and developmental abnormality	50	5.9
GIT torsion/obstruction	35	4.1
GIT ulcer/perforation/foreign body	25	3.0
Diagnosis not reached	24	2.8
Peritonitis	18	2.1
Integument/Musculoskeletal	13	1.5
Unclassified	11	1.3
Cardiac/circulatory conditions	9	1.1
Fractures/Calving injuries	8	1.0
Liver disease	5	0.6
Urinary Tract conditions	5	0.6
Bovine Neonatal Pancytopaenia	4	0.5
CNS	4	0.5
Trauma	4	0.5
Abscessation	3	0.4
BVD/Mucosal disease	3	0.4
Poisoning	2	0.2
Reproductive Tract Conditions	2	0.2
Clostridial disease	1	0.1
Tick Borne Fever	1	0.1

Figure 2: Diagnoses of neonatal calves. Conditions most frequently diagnosed on *post-mortem* examinations of bovine neonatal calves in 2018 (n= 845).

Figure 3: Septal defect. Enlarged liver, ascites (arrow) and pulmonary oedema observed in chronic passive congestion as result of a congenital ventricular septal defect (arrow in left inset). Right inset: Accentuated hepatic lobular pattern (nutmeg liver) due to chronic passive congestion. Photo: Cosme Sánchez-Miguel.

Navel ill/ joint ill has stayed at a level consistent with previous years at 7.7 *per cent*. *Escherichia coli* and *Trueperella pyogenes* were the infectious agents most frequently isolated. Similarly, the rate of peritonitis cases has stayed at 2.1 *per cent*, with *Trueperella pyogenes* and *Escherichia coli* commonly isolated from such cases.

Respiratory infections are normally responsible for about one in ten deaths in neonatal calves, and at 11.5 *per cent*, 2018 was a typical year.

Nutritional and metabolic conditions equate to 6.3 *per cent* of diagnoses in neonatal calves. This category includes failure of passive transfer of humoral immunity (hypogammaglobulinaemia) and ruminal milk drinking.

Hereditary and developmental abnormalities were recorded in DAFM laboratories in almost 6 *per cent* of carcasses submitted. Common diagnoses in this category include intestinal atresia and cardiac defects. Cardiac abnormalities most commonly noted were ventricular and atrial septum defects and persistent patent foramen ovale (Figure 3).

Calves (one to five months of age)

Respiratory infections are by far the biggest cause of mortality among 1–5 *month* old calves in Ireland (Table 2 & Figure 4). They accounted for 34.3 *per cent* of deaths in this age group. Examination of data for the last few years shows that respiratory infections are

responsible for an increasing percentage of deaths in this age category, though this has leveled off recently. The percentage of respiratory infections in this age category has risen by approximately 8 *per cent* since 2014. A breakdown of detected agents in these cases is presented in the *Bovine Respiratory Disease* section of this report.

Table 2: Conditions most frequently diagnosed on *post-mortem* examinations of calves (1-5 months old) in 2018 (n= 669)
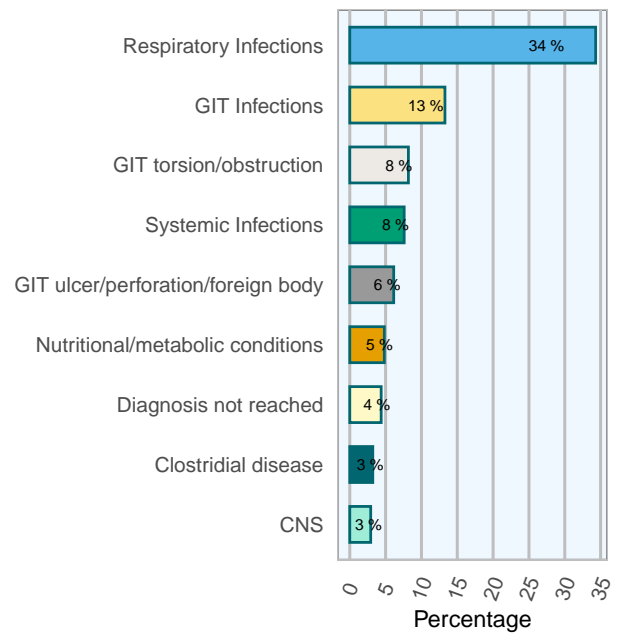
Category	No. of Cases	Percentage
Respiratory Infections	235	34.3
GIT Infections	91	13.3
GIT torsion/obstruction	56	8.2
Systemic Infections	52	7.6
GIT ulcer/perforation/foreign body	42	6.1
Nutritional/metabolic conditions	33	4.8
Diagnosis not reached	30	4.4
Clostridial disease	22	3.2
CNS	20	2.9
Poisoning	14	2.0
Navel Ill/Joint Ill	13	1.9
Peritonitis	13	1.9
Cardiac/circulatory conditions	12	1.8
Urinary Tract conditions	12	1.8
Hereditary and developmental abnormality	9	1.3
Tuberculosis	8	1.2
Integument/Musculoskeletal	7	1.0

Gastro-intestinal tract (GIT) infections (13.3 per cent) and systemic infections (7.6 per cent) remain similar to the trend of previous years as the second and third most frequently diagnosed conditions accounting for one in five of diagnosed causes of death. Common bacterial agents implicated include *Salmonella enterica Dublin* and *Escherichia coli*. Coccidia (*Eimeria spp.*) are the most frequently detected GIT pathogens in this age group.

Navel ill/ joint ill, consequences of navel infections at birth, were diagnosed in 1.9 *per cent* of calves in this age group presented to DAFM laboratories. Diagnosis of peritonitis in this age category has remained at a consistent level, between 1 and 3 *per cent*, from 2014 to 2018.

GIT ulcers and perforations continued to be a frequent diagnosis in 2018 accounting for 8.2 *per cent* of diagnoses. Perforating abomasal ulcers, leading to leakage of stomach contents and peritonitis, accounted for the majority of these cases (Figure 5).

GIT torsion/obstruction was recorded in 8.2 *per cent* of calves. Torsions of intestines, full mesentery, abomasum, omasum and reticulum were recorded. There was

Figure 4: Diagnoses of calves. Conditions most frequently diagnosed on *post-mortem* examinations of calves (1–5 months old) in 2018 (n= 669)

no fluctuation in their occurrence from previous years. Nutritional and metabolic conditions were diagnosed in 4.8 *per cent* of calves. The leading diagnoses in this category were ruminal acidosis and malnutrition.

Figure 5: Fibrinous peritonitis. Peritonitis resulting from a perforating abomasal ulcer (inset). Fatal Photo: Cosme Sánchez-Miguel.

Weanlings (six months to one year of age)

As in previous years, respiratory infections were the most commonly diagnosed cause of mortality (32.3 *per*

cent) in this age group (Table 3 & Figure 7).

GIT infections were identified as the second most common cause of death in six to 12 month-old weanlings in Ireland at 21.6 *per cent*.

Clostridial diseases were the third biggest grouping of cause of mortality in this age group (8.7 *per cent*).

Figure 6: Clostridial myositis (blackleg). Muscular necrosis and oedema in the gluteus muscle of a weanling. Photo: Cosme Sánchez-Miguel.
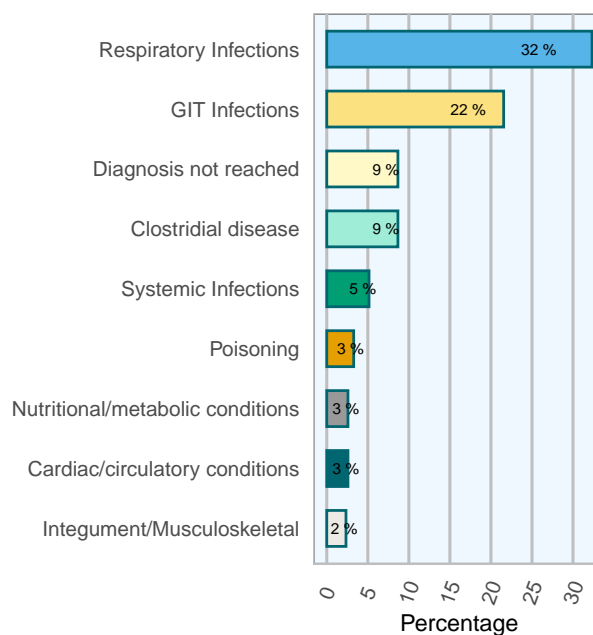
Diseases of the central nervous system (CNS) were diagnosed in 2.1 *per cent* of carcases in Ireland in 2018. Diseases in this category include cerebro-cortical necrosis, encephalopathies, encephalitis/meningitis and thrombotic meningo-encephalitis.

Table 3: Conditions most frequently diagnosed on *post-mortem* examinations of weanlings (6–12 months old) in 2018 (n= 396)

Category	No. of Cases	Percentage
Respiratory Infections	138	32.3
GIT Infections	92	21.6
Clostridial disease	37	8.7
Diagnosis not reached	37	8.7
Systemic Infections	22	5.2
Poisoning	14	3.3
Cardiac/circulatory conditions	11	2.6
Nutritional/metabolic conditions	11	2.6
Integument/Musculoskeletal	10	2.3
CNS	9	2.1
Tuberculosis	9	2.1
GIT torsion/obstruction	6	1.4

Figure 7: Diagnoses in weanlings. Conditions most frequently diagnosed on *post-mortem* examinations of weanlings (6–12 months old) in 2018 (n= 396)

Table 4: Conditions most frequently diagnosed on *post-mortem* examinations of adult cattle (over 12 months old) in 2018 (n= 527)

Category	No. of Cases	Percentage
Respiratory Infections	85	15.7
Diagnosis not reached	83	15.3
Cardiac/circulatory conditions	47	8.7
GIT Infections	32	5.9
Nutritional/metabolic conditions	32	5.9
Clostridial disease	29	5.3
Peritonitis	28	5.2
Poisoning	26	4.8
CNS	22	4.0
GIT ulcer/perforation/foreign body	22	4.0
Systemic Infections	22	4.0
Urinary Tract conditions	13	2.4
Integument/Musculoskeletal	12	2.2
Reproductive Tract Conditions	11	2.0
Liver disease	10	1.8
Babesiosis	9	1.7
GIT torsion/obstruction	9	1.7
Tumour	8	1.5
Unclassified	8	1.5
Abscessation	7	1.3
Johne's Disease	6	1.1
Tuberculosis	6	1.1

Adult Cattle (over 12 months of age)

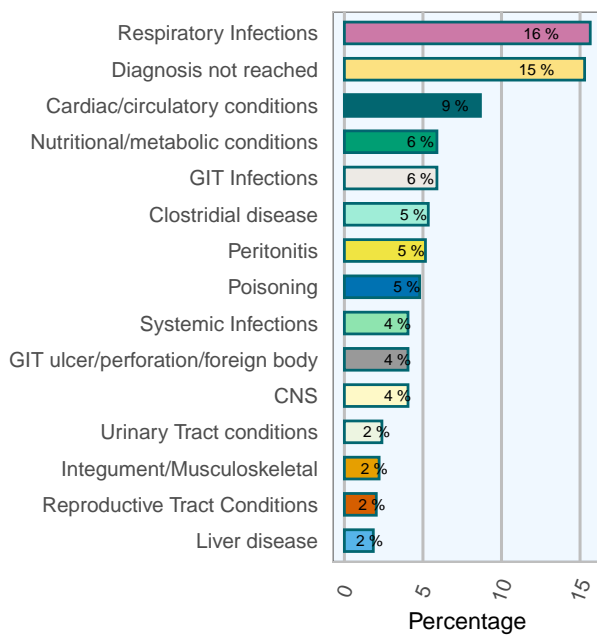Similar to previous years, respiratory diseases accounted for 15.7 *per cent* of adult deaths, (Table 4 & Figure 8). This incidence has remained roughly static in Ireland since 2014.

Figure 8: Diagnoses of adult cattle. Conditions most frequently diagnosed on *post-mortem* examinations of adult cattle (over 12 months old) in 2018 (n= 527)

Cardiac/circulatory system conditions were the second biggest diagnosis in adult cattle in Ireland, occurring in 8.7 *per cent* of cases. Endocarditis, pericarditis, caudal vena cava thrombosis, haemorrhage and haemolysis were common diagnoses in this category. *T. pyogenes* was regularly isolated from cases of endocarditis, pericarditis and caudal vena cava thrombosis.

Clostridial disease only accounted for 5.3 *per cent* of adult cattle deaths diagnosed and included cases of blackleg (Figure 6), malignant oedema, botulism and tetanus. Cases of GIT ulceration/perforation and foreign body accounted for 4.3 *per cent* of deaths, a slight decrease on rates in previous years. Hardware disease or traumatic reticuloperitionitis account for a significant proportion of these cases every year. Peritonitis diagnoses equated to 5.2 *per cent* in Ireland, in keeping with the trend of previous years.

References

DAFM (2016). "All-island Animal Disease Surveillance Report." URL https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/labservice/rvlreports/AIDSRReport016230118.pdf.

Johne's Disease

Aideen Kennedy[a]

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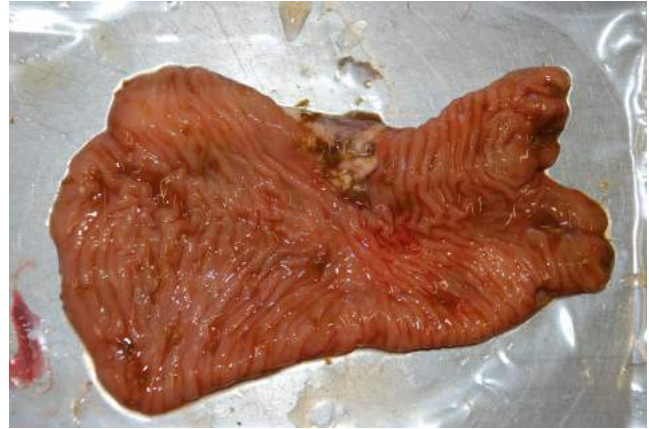
Johne's disease (JD) is a chronic granulomatous enteritis of ruminants caused by _Mycobacterium avium subspecies paratuberculosis_ (MAP). Clinical JD is characterised by diarrhoea and progressive cachexia and ultimately results in death.

In total, seventy seven positive MAP faecal cultures, from 53 different herds, were recorded in 2018 (Figure 2). Ten herds had more than one positive culture, with seven being the highest number of MAP faecal positive cultures in one single farm. Over 90 _per cent_ of positive animals were female. A mixture of dairy and beef breeds recorded positive results. However, almost 30 _per cent_ of positive samples were from Holstein Friesians (Table 1).

Table 1: Summary of 2018 MAP positive faecal cultures by breed and gender

Breed	Female	Male	Total
Holstein Friesian	22 (31.0)	1 (16.7)	**23 (29.9)**
Limousin	12 (16.9)	1 (16.7)	**13 (16.9)**
Charolais	9 (12.7)	0 (0.0)	**9 (11.7)**
Friesian	8 (11.3)	0 (0.0)	**8 (10.4)**
Jersey	8 (11.3)	0 (0.0)	**8 (10.4)**
Aberdeen Angus	4 (5.6)	3 (50.0)	**7 (9.1)**
Belgian Blue	3 (4.2)	0 (0.0)	**3 (3.9)**
Shorthorn	1 (1.4)	1 (16.7)	**2 (2.6)**
Blonde D'Aquitaine	1 (1.4)	0 (0.0)	**1 (1.3)**
Hereford	1 (1.4)	0 (0.0)	**1 (1.3)**
Norwegian Red	1 (1.4)	0 (0.0)	**1 (1.3)**
Partanaise Cross	1 (1.4)	0 (0.0)	**1 (1.3)**

Latency is a common feature of mycobacterial diseases, animals can remain sub-clinically infected without showing any clinical signs of the disease for many years. Clinical disease is reported to occur most frequently in cattle aged 2–5 _years_. In line with this, based on a number of assumptions, it is estimated that 50 _per cent_ of animals that tested positive in 2018 were displaying symptoms of JD by four years of age (Figure 3). At time of analysis, all male animals that had a positive result in 2018 were no longer alive and only 24 _per cent_ of positive female animals were still alive (Figure 4).

Figure 1: Thickened and wrinkled intestinal mucosa (granulomatous enteritis) in the ileum of a cow with Johne's disease (_Mycobacterium avium ssp. paratuberculosis_). Photo: Cosme Sánchez-Miguel.

JD transmission

In many herds, initial introduction of MAP usually occurs as result of acquiring an infected but clinically normal animal. In 2018, a number of animals that subsequently recorded MAP faecal culture positive results underwent multiple herd movements throughout their lifetime, potentially allowing spread of the disease. Five was the greatest number of herd movements recorded by a positive animal, excluding movements to factory or knackery (Table 2).

Table 2: Movement statistics excluding movements to factory or knackery.

Minimun	Median	Maximun
0	1	5

Once MAP is introduced to a herd, infection with MAP is understood to occur, primarily, as a calf. Animals younger than six months are believed to be the most susceptible. Neonates are considered to be at highest risk of acquiring MAP infection due to increased permeability of intestines during the first 24 _hours_ of life and an immature immune system. Older animals are believed to be less susceptible; however, infection can still occur (Windsor and Whittington, 2010).

Figure 2: Number of herds by county with at least one animal diagnosed with JD by faecal mycobacterial culture in 2018.

Figure 3: Survival curves measure how much time elapsed before a certain event occurred. In this case, the event is represented by submission of a faecal sample to an RVL. An assumption is made that faecal samples are submitted soon after the animal displays diarrhoea unresponsive to treatment. 50 % of animals may have displayed symptoms consistent with the disease by four years of age. The graph on the bottom represents number of animals at risk of developing symptoms over time.

Severity and rate of JD progression in individual animals are dependent on MAP exposure dose and age at time of infection. Infection usually occurs via the faecal-oral route, although in-utero transmission can also occur. Exposure of calves to adult faeces is the most important risk factor in MAP transmission (Doré *et al.*, 2012). Faecal-oral transmission is facilitated by faecal contamination of feedstuff and calf's environment, with highest environmental risk factors for neonatal infection being faecal contamination of udders and calving pens. Colostrum and milk from infected cows can also contain quantities of MAP capable of infecting calves. Feeding pooled colostrum or milk from multiple cows of unknown MAP status is considered to increase risk of infection within a herd.

JD Diagnostics

As treatment of MAP is generally regarded as ineffective, diagnostic testing is often used to direct subsequent management decisions (e.g. calf in separate area, cull, etc.) and allow preventative management measures of non-infected herd mates. As MAP is a slow growing bacterium, infection can remain latent for many years making diagnosis difficult. Diagnostic tests

Figure 4: Status of animals diagnosed in 2018 with Johne's disease as per the 25th of April, 2019

Figure 5: Microphotography of Langhan's-type giant cells (arrows) occasionally observed in tuberculoid granulomas seen in the lamina propia of the small intestine in animals with Johne's disease. Inset: Ziehl-Neelsen stained section showing acid-fast (*Mycobacterium avium ssp. paratuberculosis* bacilli). Photo: Cosme Sánchez-Miguel.
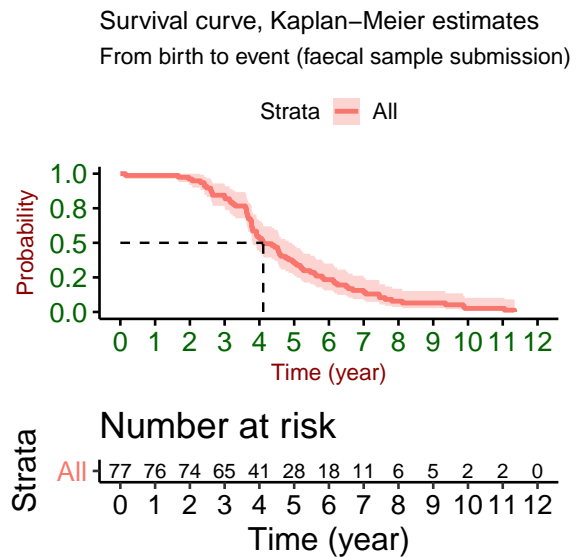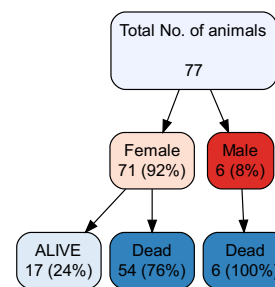
currently in use involve either identification of MAP itself (culture), identification of MAP genetic elements (PCR), or detection of the immune response MAP infection elicits (ELISA) (Behr and Collins, 2010).

Faecal culture is generally taken as the reference test for MAP. An advantage of culture is that detection of MAP in faecal samples confirms presence of viable MAP in an animal. Due to absent or intermittent shedding of bacteria early in the disease process, sensitivity of culture can be low. Specificity, however, is almost 100 *per cent*. Due to the fastidious nature of MAP, culture takes a number of weeks. Polymerase Chain Reaction (PCR) is another faecal based test used to detect DNA of MAP, it offers a rapid method of detecting MAP status.

Enzyme Linked Immune Sorbent Assay (ELISA) examines the host's immune response to MAP and is extensively used for routine diagnosis. ELISA is favoured as a screening test due to its relatively low cost, compared to faecal culture or PCR. ELISAs also provide faster results when compared to culture methods. ELISA relies on identifying serum antibodies to a particular antigen as an indicator of infection. It is important to note that a positive ELISA reaction is NOT confirmation of JD. The specificity of MAP ELISA tests can be influenced by tuberculin testing and by exposure to non-MAP environmental mycobacteria (giving rise to false positive results). The sensitivity of MAP ELISA tests is influenced by stage of infection, high in animals with clinical disease but low in infected animals that are shedding few MAP organisms (where false negative results may arise).

Post mortem examination

On post mortem, gross and microscopic lesions associated with JD are primarily confined to the intestine and mesenteric and ileo-caecal lymph nodes. Gross lesions are characterised by thickening and corrugation of intestinal mucosa, most prominent in distal ileum and ileo-caecal valve. Histological lesions associated with JD can vary widely; villi are frequently fused and mucosa is invariably thickened, infiltration of macrophages -including giant cells- is commonly identified in the submucosa and acid fast bacilli are commonly present. JD cannot be diagnosed solely on post mortem, diagnosis needs to be confirmed by faecal culture and/or histology (intestine/lymph nodes).

Control Programme

A voluntary national JD control programme is on-going in Ireland under the guidance of Animal Health Ireland. The aim is to provide pathways for test-negative and test-positive herds to demonstrate progress towards an improved herd assurance for JD. Primary aspects of this programme involve identification of potentially infected animals via either milk or blood ELISA testing, confirmation relying on faecal based testing. Highlighting on farm management practices using veterinary risk assessment and management plans (VRAMP) is commonly used as a tool in a number of control programmes, including AHI's JD control programme. VRAMP is a combined work between a farmer and a trained local vet familiar with his/her farm which facilitates identifying specific high risk management practices occurring in such farm that may facilitate spread of JD. Repeat visits allow monitoring of successful implementation of management changes.

References

Behr MA, Collins DM (2010). *Paratuberculosis: organism, disease, control.* CABI.

Doré E, Paré J, Côté G, Buczinski S, Labrecque O, Roy J, Fecteau G (2012). "Risk Factors Associated with Transmission of Mycobacterium avium subsp. paratuberculosis to Calves within Dairy Herd: A Systematic

Review." *Journal of Veterinary Internal Medicine*, **26**(1), 32–45. doi:https://doi.org/10.1111/j.1939-1676.2011.00854.x. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1939-1676.2011.00854.x, URL https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1939-1676.2011.00854.x.

Windsor PA, Whittington RJ (2010). "Evidence for age susceptibility of cattle to Johne's disease." *The Veterinary Journal*, **184**(1), 37 – 44. ISSN 1090-0233. doi:https://doi.org/10.1016/j.tvjl.2009.01.007. URL http://www.sciencedirect.com/science/article/pii/S1090023309000094.

Bovine and Ovine Clostridial Diseases

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Clostridial spp. cause significant disease in both sheep and cattle and are encountered regularly in post mortem room submissions. They also cause disease in other species including goats and pigs. Typically, clostridial disease presents as acute disease or sudden death and mortality approaches 100% in most cases.

Blackleg

Blackleg is the most frequently diagnosed clostridial disease in bovine submissions; it is commonly, but not exclusively, associated with detection of *Clostridium chauvoei* (Table 1 and Figure 3). The pathogenesis of this disease requires pre-existence of bacteria in tissue that, in conjunction with favourable circumstances such as trauma, establish anaerobic conditions which allow bacterial proliferation and toxin production, the latter causes severe local necrotising myositis (Figure 1) and systemic toxaemia. Cases encountered in *post-mortem* rooms frequently have a typical rancid butter odour and affect muscles of the limbs, tongue and heart.

Figure 1: Blackleg lesion (severe necrotising myositis) in the hindquarters of a bullock. Photo: Maresa Sheehan.

Botulism

Botulism is the second most frequently diagnosed bovine clostridial disease. *Clostridium botulinum* toxin typically results in affected animals lying in sternal recumbancy with the head on the ground or turned into

Table 1: Clostridial disease diagnosed in bovine carcasses in 2018 (n= 88).

Disease	No. of Cases	Percentage
Blackleg	40	**46**
Botulism	21	**24**
Malignant Oedema	14	**16**
Enterotoxaemia	10	**11**
Black Disease	3	**3**

the flank, similar to a cow suffering from post parturient hypocalcaemia/milk fever. However, a range of clinical signs can be detected within an affected group, likely reflecting levels of toxin ingested, these can include restlessness, inco-ordination and knuckling. The association of this disease with the spread of poultry litter has resulted in Codes of Practice being established for disposal of such material. The laboratory service has been involved in the investigation of a number of cases where a direct link with poultry litter was not established, carrion and forage associated botulism could not be ruled out in these cases.

Malignant Oedema

Malignant oedema can be caused by a number of *Clostridial spp.* including *C. septicum, chauvoei, sordellii* and *novyi*. Epidemiology and pathogenesis of this disease differs from blackleg in that bacteria is introduced through a wound and causes focally extensive skin gangrene and oedema of the sub-cutaneous and intra-muscular connective tissue, there is less frequent involvement of underlying muscle.

Enterotoxaemia

Enterotoxaemia is a disease caused by *Clostridium perfringens* that causes significant losses in both cattle and sheep (Table 2 and Figure 5). This micro-organism can be a normal inhabitant in the intestine of most species inlcuding humans. When intestinal environment is altered by sudden changes in diet or other factors, *C. perfringens* proliferates in large numbers and produces several potent toxins that are absorbed into the general circulation or act locally with usually devastating

Figure 2: Perivascular oedema in the brain associated with vascular compromise due to Epsilon toxin-Pulpy kidney. Photo: Maresa Sheehan.

Figure 3: Clostridial disease diagnosed in Bovine carcasses in 2018 (n= 88).
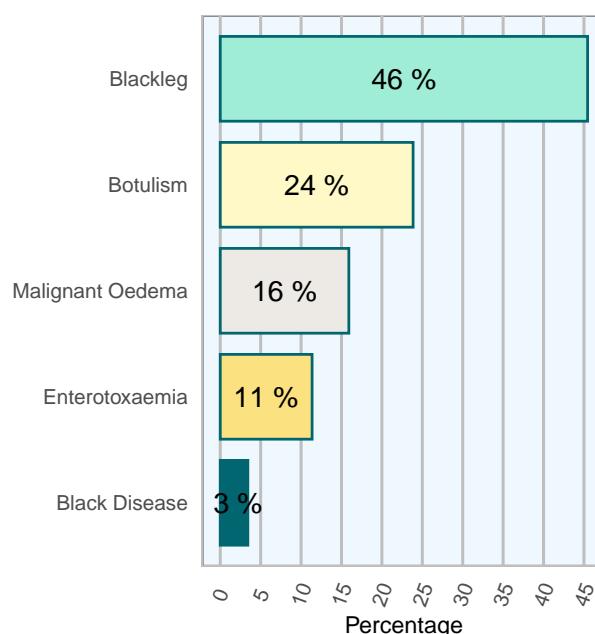
effects on the host. Enterotoxaemia diagnosis presents a diagnostic challenge as this bacteria is a normal inhabitant of the gut, therefore, demonstration of toxins is essential. However, as some of the toxins can be present in small amounts in clinically normal animals, presence of concurrent gross lesions and histopathological changes is desirable.

Table 2: Clostridial disease diagnosed in ovine carcasses in 2018 (n= 101).

Disease	No. of Cases	Percentage
Pulpy Kidney Disease	38	**38**
Enterotoxaemia	36	**36**
Malignant Oedema	12	**12**
Abomasitis- emphysematous	8	**8**
Black Disease	6	**6**
Braxy	1	**1**

In lambs, *C. perfringens* type A produces a rare form of acute enterotoxemia known as *yellow lamb disease*, clinically characterised by depression, anemia, icterus and hemoglobinuria (Uzal and Songer, 2008). In cattle, it has been associated with haemorrhagic enteritis, indistinguishable from that caused by Types B and C. It has also been proposed as a cause of acute deaths in calves due to clostridial abomasitis and of jejunal haemorrhage syndrome in adult cows. The latter is a disease entity sporadically diagnosed in the veterinary laboratory service and, consequently, no definitive con-

clusions on its aetiology can be made (Van Kruiningen *et al.*, 2009; Songer and Miskimins, 2005).

Clostridium Perfringens Type B and C can cause sudden death, with or without haemorrhagic enteritis, in lambs and calves, and *struck* in adult sheep. *Clostridium perfringens* Type D produces epsilon toxin, which causes vascular endothelial damage (Figure 2) resulting in typical lesions of *Focal Symmetrical Encephalomalacia (FSE)* and *pulpy kidney*. In the PM room, detection of fibrin clots in fluid of the pericardial sac (Figure 4) is a strong indicator of epsilon toxin involvement.

Black Disease

Black Disease is a less frequently diagnosed cause of acute or sudden death in cattle and sheep. *Clostridium novyi* proliferates in anaerobic conditions, typically associated with liver damage due to *Fasciola hepatica* migration. Multifocal areas of hepatic necrosis are observed at post mortem. Pathogenesis of *bacillary haemoglobinuria* is similar, although in addition to multifocal hepatic necrosis and vascular damage, the organism also produces an haemolytic toxin which causes haemoglobinuria. *Clostridium haemolyticum* should be included in the list of differentials for haemoglobinuria, which also includes *Babesiosis, periparturient hypophosphataemia, copper poisoning* in sheep and *brassica poisoning* amongst others.

Figure 4: Fibrin clot in pericardial sac associated with clostridial enterotoxaemia. Photo: Maresa Sheehan.
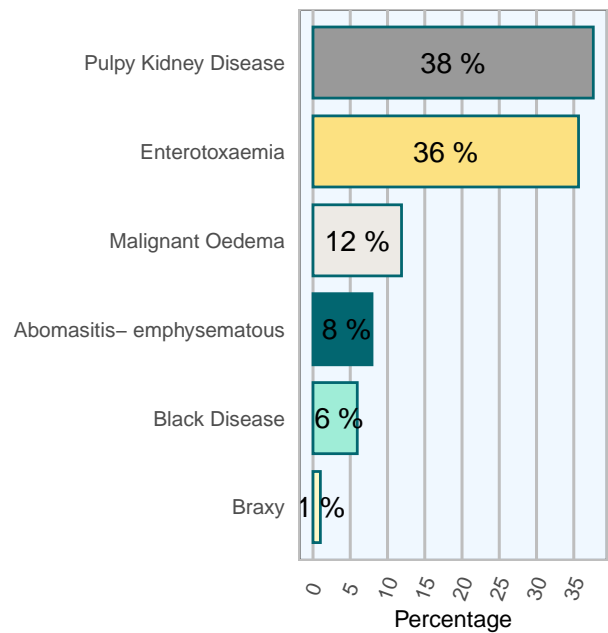
Braxy

Braxy is caused by *Clostridium septicum* and is typically seen in animals submitted after grazing frozen/snow covered pasture. It causes necrosis, ulceration, congestion and emphysema of the abomasal wall.

Clostridial abomasitis

Abomasitis caused by *Clostridium sordellii* has a similar post mortem presentation. Histopathology will reveal a severe necrotising abomasitis (Figure 6) with intra-lesional bacilli. It is essential that gross signs and histopathology concur with isolation/FAT detection of bacteria, as *C. sordellii* and *septicum* are normal inhabitants of the gut.

Figure 6: Haemorrhagic and emphysematous abomasitis associated with Clostridium sordellii. Photo: Maresa Sheehan.

Figure 5: Clostridial diseases diagnosed in Ovine carcasses in 2018 (n= 101).

Clostridial Vaccination

Vaccination using a multivalent vaccine is recommended for protection of animals against clostridial diseases. Due to the ubiquitous nature of agents involved, vaccination should be considered an essential component of herd/flock management. However, in a recent study into sheep mortality by regional veterinary laboratories, fifteen flocks that reported vaccinating against clostridial disease recorded a clostridial disease diagnosis. This finding is not wholly unexpected as vaccination of flocks does not infer *sterile immunity*, nor does detection of a pathogen on postmortem necessarily infers 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 clostridial disease in lambs may be due to insufficient maternal transfer of immunity to newborn lambs or waning of passive immunity in older lambs. Uzal and Songer (2008) and Songer and Miskimins (2005) reported that, although widely used, there can be variations in individual responses or manufacturer's vaccine quality, when determining the response of sheep flocks to multivalent clostridial vaccination (Murray *et al.*, 2019).

References

Murray GM, Fagan S, Murphy D, Fagan J, Ó Muireagáin C, Froehlich-Kelly R, Barrett DJ, Sheehan M, Wilson M, Brady CP, Hynes F, Farrell S, Moriarty J, O Neill R, Casey M (2019). "Descriptive analysis of ovine mortality in sentinel sheep flocks in Ireland." *Veterinary Record*, **184**(21), 649–649. ISSN 0042-4900. doi:10.1136/vr.105291. https://veterinaryrecord.bmj.com/content/184/21/649.full.pdf, URL https://veterinaryrecord.bmj.com/content/184/21/649.

Songer JG, Miskimins DW (2005). "Clostridial abomasitis in calves: Case report and review of the literature." *Anaerobe*, **11**(5), 290 – 294. ISSN 1075-9964. doi:https://doi.org/10.1016/j.anaerobe.2004.12.004. URL http://www.sciencedirect.com/science/article/pii/S1075996405000430.

Uzal FA, Songer JG (2008). "Diagnosis of Clostridium perfringens intestinal infections in sheep and goats." *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*, **20**, 253–265. ISSN 1040-6387. doi:10.1177/104063870802000301.

Van Kruiningen HJ, Nyaoke CA, Sidor IF, Fabis JJ, Hinckley LS, Lindell KA (2009). "Clostridial abomasal disease in Connecticut dairy calves." *The Canadian veterinary journal = La revue veterinaire canadienne*, **50**, 857–860. ISSN 0008-5286.

Bovine Neonatal Enteritis

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Neonatal enteritis is consistently the most frequently diagnosed cause of mortality in calves less than one month old in the Republic of Ireland. It is generally caused by the interaction of one or more infectious enteric pathogens and several predisposing factors.
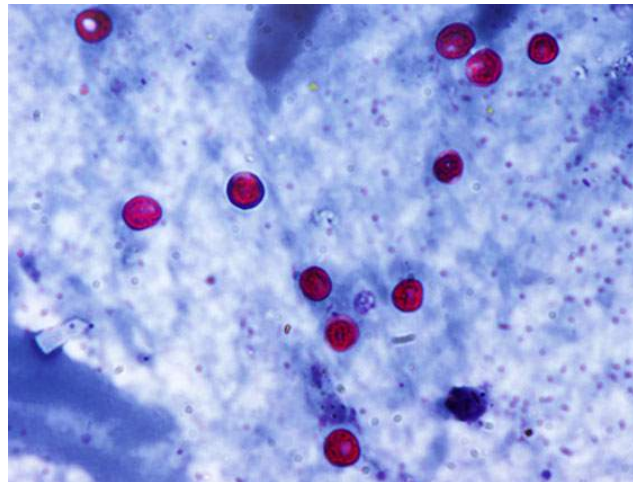
Neonatal enteritis

The most common clinical presentation in neonatal enteritis is watery diarrhoea (occasionally containing blood) usually leading to dehydration and, in severe cases, progressing to profound weakness and death. In order to identify the enteric pathogens involved in cases of neonatal calf diarrhoea, a series of tests are performed on faecal samples collected at *post mortem* from affected calf carcases or submitted by veterinary practitioners from clinical cases.

Approximately 1800 such faecal samples were examined in DAFM laboratories in 2018 (Table 1). This section shows the most common infectious agents diagnosed in calves less than one month old. The relative frequency of identification of pathogens in calf faecal samples in the Republic of Ireland in 2018 is plotted in Figure 2. Rotavirus and *Cryptosporidium spp.* were the most common pathogens identified while *E coli* K99, coronavirus and *Salmonella* Dublin were recorded relatively infrequently.

Table 1: Number of tests and relative frequency of enteropathogenic agents identified in faecal samples of calves up to one month of age in 2018.

Organism	No. of Tests	Positive	Percentage
Rotavirus	1757	599	**34.1**
Cryptosporidia	1871	413	**22.1**
Campylobacter Jejuni	1644	146	**8.9**
Giardia	1080	87	**8.1**
Coronavirus	1763	23	**1.3**
E.Coli K99	1299	15	**1.2**
Salmonella Dublin	1756	16	**0.9**

Rotavirus enteritis. Rotavirus (34.092202618099 *per cent*) has consistently been the most frequently identified pathogen in calf faecal samples in the Republic of Ireland in recent years, its frequency ranging from 30–35 *per cent* between 2010 and 2018 (Figure 4). Calves

Figure 1: Cryptosporidial oocysts in a faecal smear, modified Ziehl-Neelsen (Z-N) stain. Photo: Cosme Sánchez-Miguel.

are most susceptible to rotavirus enteritis up to three weeks of age. Adult animals are the primary source of rotavirus infection for neonatal calves. The severity of clinical signs depends on several factors including the age of the animal and the immune status of the calf, the latter depends on the absorption of colostral antibodies immediately after birth. Rotavirus targets villi in the upper small intestine causing shortening and fusion of such villi, this results in malabsorption and leads to diarrhoea. Death may ensue due to acidosis, dehydration and starvation.

Cryptosporidiosis. *Cryptosporidium parvum* is a small single-cell parasite which causes damage to intestinal epithelial cells of the lower small intestine resulting in mild to severe enteritis, typically affecting calves during their second week of life. Affected calves excrete large numbers of oocysts which are highly resistant and can survive in the environment up to several months under favourable conditions. Transmission between animals is by faecal-oral route, often via a contaminated environment. Control of the parasite is best achieved by strict maintenance of good calf housing hygiene practices and avoiding mixing animals of different ages. Ammonia-based disinfectants are most effective. The prophylactic use of drugs such as halofuginone lactate

Figure 2: Bovine Neonatal Enteritis. Relative frequency of enteropathogenic agents identified in calf faecal samples tested in 2018.

may also be useful. In addition to causing disease in animals, *Cryptosporidium spp.* has the potential to cause zoonotic disease especially in immunocompromised humans; therefore farm workers should take appropriate hygiene precautions when handling calves.

Coronavirus enteritis. Calves are most susceptible to coronavirus enteritis between one and three weeks of age. Coronavirus preferentially infects enterocytes in the lower small intestines and colon, typically resulting in blunting and fusion of villi and mild enterocolitis.

***Escherichia coli* K99.** *E. coli* K99 is an enterotoxigenic *E. coli* (ETEC) and an important cause of neonatal enteritis in very young calves, typically less than three days of age. These strains of E. coli preferentially colonise the lower small intestine and produce toxins that cause secretion of water and electrolytes from the intestinal mucosa, resulting in rapid dehydration. The percentage prevalence of *E. coli* K99 would likely be higher if testing for this enteric pathogen was restricted to animals younger than one week old.

***Salmonella* Dublin.** *Salmonella enterica* subsp.*enterica* serovar Dublin (*Salmonella* Dublin) is the most common *Salmonella* serotype that affects calves

in the Republic of Ireland and was isolated in 0.911161731207289 *per cent* of neonatal faecal samples cultured in 2018. The relative frequency of detection of *Salmonella* Dublin from such cases has fallen significantly over the past decade from 3.4 *per cent* in 2011 to 0.9 *per cent* in 2018 (Figure 4). It is not clear why this has occurred. *Salmonella* Dublin infection has a number of clinical presentations in neonatal calves including acute enteritis, osteomyelitis and septicaemia/systemic disease. *Salmonella* enteritis is characterised by watery mucoid diarrhoea with presence of fibrin and blood. While Salmonella can cause diarrhoea in both adult cattle and calves, infection is more common and often more severe in calves from 10 days to 3 months old. In addition, calves can shed the organism for variable periods of time and/or intermittently depending on the degree of infection (carrier state).

Prevention and control of neonatal enteritis

The basic principles for the prevention and control of neonatal enteritis are enhancing host immunity and reducing the load of enteric pathogens in the environment. The importance of good colostrum management, leading to an adequate passive immunity transfer, in the prevention of calf diarrhoea is a given.

An average 40 *Kg* calf requires 3 *litres* of colostrum within 2–4 *hours* of birth. Thereafter, appropriate nutrition of young calves, including diarrhoeic calves, is essential. Calves should be grouped according to age on dry clean bedding and avoiding high stocking density. There should be good hygiene practices including appropriate disinfection between batches and rapid isolation and treatment of sick calves. Colostral and milk antibodies against certain bacterial and viral enteric agents can be enhanced by vaccination of the cows during the dry period (passive immunisation).

Campylobacter jejuni. *C. jejuni* was found in almost 8.88077858880779 *per cent* of neonatal calf faecal samples tested in 2018. It is not considered pathogenic for cattle. However, it is routinely surveyed in neonatal faecal samples because it is a zoonosis and a major cause of gastroenteritis in humans. Therefore, appropriate

Figure 3: Microphotography of coccidial oocysts in a faecal smear (faecal flotation). Photo: Cosme Sánchez-Miguel.

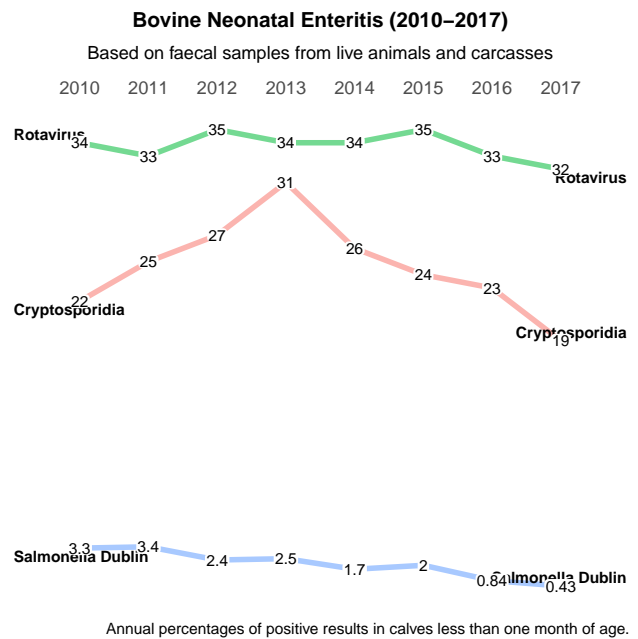hygiene precautions should be taken by personnel handling stock.

Giardia spp.. *Giardia spp.* is one of the most prevalent and widespread intestinal protozoan parasite in humans and several vertebrate animal species worldwide. The clinical significance of *Giardia spp.* as enteric pathogen in calves is questionable. While eight strains are recognized, only two strains are thought to be transferable to humans (A&B) and thus potentially zoonotic (Thompson, 2004). Appropriate precautions should be taken by calf handlers.

Coccidiosis. Coccidiosis is caused by protozoan parasites of the genus *Eimeria spp.* Only three (*Eimeria bovis*, *Eimeria alabamensis* and *Eimeria zuernii*) out of twelve bovine coocidia species are pathogenic. Some of the non-pathogenic or weakly pathogenic species are capable of producing massive numbers of oocysts, therefore faecal coccidial occyst counts need to be interpreted in conjunction with history and clinical findings. Coccidia damage the epithelial cells lining of the gut causing diarrhoea and possibly dysentery.

Table 2: Number of tests and relative frequency of coccidiosis in faecal samples of calves up to one month of age in 2018.

No. of Tests	Positive	Percentage
610	107	**18**

Coccidiosis is particularly common in calves between

Bovine Neonatal Enteritis (2010–2017)

Based on faecal samples from live animals and carcasses

Annual percentages of positive results in calves less than one month of age.

Figure 4: Trends in the incidence of Rotavirus, Cryptoporidia and *Salmonella* Dublin in calves less than one month old with neonatal enteritis.

three weeks and six months of age but it can occur in older animals also. Calves become infected when placed in environments contaminated by older cattle or other infected calves, e.g. indoors on bedding, outdoors around drinking and feeding troughs. Poor hygiene, high stocking density and poor health and nutrition will all contribute to a calf becoming infected. The frequency of detection of coccidiosis in neonatal calves less than 1 month old in the Republic of Ireland in 2018 was 18 *per cent* (Table 2).

> *Coccidia spp.*
>
> Often, peak coccidia oocyst-shedding does not correlate with the onset of diarrhoea in calves. Detection of *Coccidia spp.* in faecal samples is facilitated by sampling pre-clinical comrade animals as well as those showing clinical signs.

References

Thompson RA (2004). "The zoonotic significance and molecular epidemiology of Giardia and giardiasis." *Veterinary parasitology*, **126**(1-2), 15–35.

Zinc Sulphate Turbidity (ZST) Test

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The Zinc Sulphate Turbidity (ZST) test is an indirect measurement of passive transfer of immunoglobulins via colostrum from the dam to the neonate. The adequate delivery of good quality colostrum is an important part of calf management, as transfer of immunity provides protection to neonates from common infectious diseases that contribute to illness and death.

ZST test and the importance of colostrum

Failure of passive transfer (FPT) is best assessed on a herd basis. It is recommended to sample several healthy calves or lambs, up to twelve animals less than a week old. Blood sampling should not be done on the first day of life as peak circulating immunoglobulin is achieved 36 hours after colostrum ingestion.

The ZST test used in the DAFM laboratory service was developed by McEwan *et al.* (1970), who determined that metal salts such as Zinc Sulphate would be precipitated from solution in proportion to the level of immunoglobulin present in a serum sample, once the two are combined. In recent years, to improve the quality of this test, a higher concentration of Zinc Sulphate solution has been used (Hudgens *et al.*, 1996).

The original cut-off point for the test was 20 *units*, which was comparable to immunoglobulin G (*IgG*) concentrations in serum of 16 *mg/ml*. *IgG* However, in years since, a concentration of IgG of 10 *mg/ml*, corresponding to a ZST result of 12.5 *units* or greater, has been considered adequate for dairy calves. It is still desirable that ZST scores reach a higher range and we have classed results greater than or equal to 20 *units* as optimal (Hogan *et al.*, 2015, 2016).

Table 1: Zinc Sulphate Turbidity Test Results in 2018 (n= 1207).

Submission type	Status	No. of samples	Mean	Percentage
Diagnostic	Optimal	588	33.0	**72**
	Adequate	120	16.4	**15**
	Inadequate	106	7.4	**13**
Carcass	Optimal	132	29.7	**34**
	Adequate	89	15.5	**23**
	Inadequate	172	6.4	**44**

Outline of 2018 figures

In 2018, 814 blood samples were submitted for Zinc Sulphate Turbidity Test for diagnosic purposes, i.e. from live animals. Table 1 and Figure 1 show that 72 *per cent* of samples submitted for diagnostic purposes were in the optimal range, i.e. had a ZST result greater than or equal to 20 *units*; 15 *per cent* were within the adequate range, ZST results between 12.5 and 20 units, and the remaining 13 *per cent* were in the inadequate range, ZST results below 12.5 units. The distribution of ZST values is charted in Figure 2.

2018 figures are a marked improvement on just a few years ago, for example in 2014 only 51 *per cent* of diagnostic samples returned a value greater than or equal to 20 units. There are two likely reasons for this improvement: information campaigns conducted by several bodies to impress upon herd-owners the importance of good colostrum management, which are likely to have led to improved colostrum feeding practices, and better targeted testing of calves to evaluate colostrum management.

> ### Serum Total Protein
>
> Measurement of serum total protein is another way to assess for failure of passive transfer (FPT). This test is useful for monitoring colostrum management in healthy calves, but it is not suitable for sick, dehydrated or dying calves. The analysis can be carried out either in farms with a refractometer (Figure 3), or in veterinary clinics using an in-house biochemistry analyser (Bielmann *et al.*, 2010). When used for screening, 80 *per cent* of samples should show values above 55 *g/l*.

Shortcomings in submission practices

The optimum way to investigate FPT is on a herd basis. Single samples are not ideal as individual results can vary and may not be reflective of herd incidence of FPT. Colostrum management should be examined on a herd basis; when assessing a herd, the proportion

of calves in the herd which have received inadequate colostral immunity is of more significance than the average serum immunoglobulin concentration.

Currently, DAFM laboratories determine the immune status of calves by ZST tests on an on-demand basis. Submissions overwhelmingly consist of a sample from a single calf; in 2018, 41 *per cent* of submissions for ZST testing contained one single sample and only 23 *per cent* of submissions contained 5 or more samples. This proportion has improved over the last few years; in 2014 single samples made up 79 *per cent* of submissions while only 7 *per cent* of submissions contained five or more samples. Awareness of the importance of colostrum in herd health, and of the need for planned investigations into the efficacy of colostrum feeding, has increased.
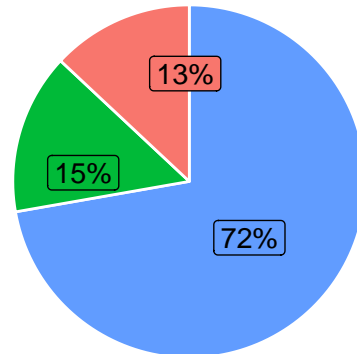
Submissions from sick calves

Clinical history provided in the laboratory submission forms is in many cases minimal but one would suspect many single samples come from sick calves. Samples from sick calves are not suitable to evaluate colostrum management as disease processes will affect circulating immunoglobulin. Immunoglobulin will be lost from circulation, as it binds with antigen, or through protein-losing conditions such as enteropathy and nephropathy. Dehydration, on the other hand, may lead to artificially high ZST results through haemoconcentration.

ZST and immunoglobulin classes

ZST tests give results which correlate well with levels of total immunoglobulin and with *IgG*, which is understandable as *IgG* comprises the largest proportion of immunoglobulin in both colostrum and the bloodstream of calves drinking colostrum. Results from the ZST test do not give as good a measure of circulating immunoglobulin M (*IgM*), which composes a smaller proportion of the immunoglobulin in colostrum and is important in the prevention of septicaemia. To complicate matters, *IgM* molecules are commonly much larger than those of *IgG* and *closure* of the intestine to *IgM*, in other words the point at which the intestinal mucosa ceases absorbing *IgM* intact into the blood stream, occurs much earlier than it does for *IgG*.

The upshot of this is that a calf receiving colostrum after a slight delay may have adequate, or even optimal,

Zinc Sulphate Turbidity Test
Diagnostic submissions

Status: ■ Inadequate ■ Adequate ■ Optimal

Figure 1: Results of ZST from submitted bovine blood samples in 2018 (n= 814).
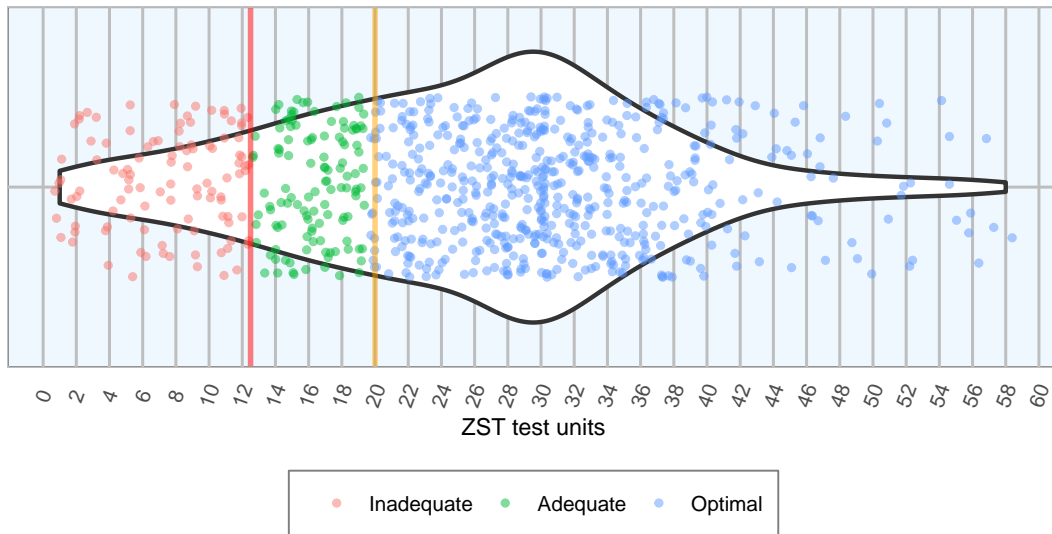
levels of *IgG* and total immunoglobulin, yet have absorbed inadequate levels of *IgM*. This inadequacy will not be reflected in ZST results or in results from any of the alternative indirect tests for FPT, such as total protein or Gamma-Glutamyl Transferase (*GGT*) levels. Only a direct test for *IgM*, such as an ELISA or radial immunodiffusion (RID), will pick up this deficiency.

Post mortem samples

Another source of samples for ZST testing in DAFM laboratories is blood harvested from calves at necropsy, which by definition are not from healthy calves. ZST results from calves sampled at necropsy, except in the case of very acute deaths, may give misleading results due to the course of illness that preceded death. However, RVL staff may use ZST results from these calves to flag possible cases of FPT in a herd, if results suggestive of FPT are returned this can prompt a possible need to investigate further the performance of colostrum management in that herd. When ZST testing was carried out on 393 samples taken from calf carcasses during *post mortem* examinations in the laboratory service, 44 *per cent* of samples had results indicating a failure of passive transfer, a further 23 *per cent* of samples were in the suboptimal range.

Violin Plot of ZST Test Results
Diagnostic submissions

ZST test units

● Inadequate ● Adequate ● Optimal

Figure 2: Distribution of ZST test results during 2018. Optimal colostral immunity is defined as greater than or equal to 20 units (orange line), adequate as equal to or greater than 12.5 and less than 20 units and inadequate as less than 12.5 units (red line). The width of the white area at each point of the x axis is proportional to the number of samples returning a ZST result of that value. Outliers greater than 60 units (24 samples) were removed from the plot n=(790).

Figure 3: Brix refractometer. Colostrum quality can be assessed by placing a drop of colostrum in the stage and looking through the eyepiece. Photo: Cosme Sánchez-Miguel.

Ovine submissions

Submissions from ovine neonates for ZST testing are low, especially diagnostic submissions of which only four were received in 2018. Of these, one was suggestive of FPT, but such a small sample size does not make it possible to draw conclusions. Samples were collected from lamb carcasses in higher numbers, and it was found that 96 out of 148 (or 65 *per cent*) were below the optimal level. Submission rates of ovine neonates for necropsy have been elevated by a study into sheep mortality which was conducted in a number of RVLs in 2018. We must again bear in mind the possible limitations of this test when performed on samples taken from dead animals. It is likely that failure of passive transfer in lambs is most commonly due to mis-mothering, the risk may be also high for lambs born as triplets.

While lambs and calves from beef breeds will usually receive adequate colostrum by suckling, dairy calves require herdowner intervention in order to consume enough colostrum to give sufficient protection. This is due to a dilution effect on colostrum quality caused by the higher volumes of milk produced by modern dairy cows. Ideally, the first feed of colostrum should be given to the calf within two hours of birth and certainly no

later than six hours after birth. The quantity required should be based on weight, with the typical 35–45 *kg* dairy calf needing 3 *l* and smaller cross-bred calves needing less. Poor transfer of colostral immunity may be due to poor quality colostrum, low colostral intake, poor colostrum absorption or a combination of these three factors.

Supplementary feeding using a stomach tube or oesophageal feeder may be necessary. Frozen colostrum may be used when necessary. Artificial colostrum is less effective but may be used as a last resort. It should always be remembered that improved colostrum feeding practices will not completely compensate for inadequate hygiene.

References

Bielmann V, Gillan J, Perkins N, Skidmore A, Godden S, Leslie K (2010). "An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle." *Journal of Dairy Science*, **93**(8), 3713 – 3721. ISSN 0022-0302. `doi:https://doi.org/10.3168/jds.2009-2943`. URL http://www.sciencedirect.com/science/article/pii/S0022030210003905.

Hogan I, Doherty M, Fagan J, Kennedy E, Conneely M, Brady P, Ryan C, Lorenz I (2015). "Comparison of rapid laboratory tests for failure of passive transfer in the bovine." *Irish veterinary journal*, **68**, 18. ISSN 0368-0762. `doi:10.1186/s13620-015-0047-0`.

Hogan I, Doherty M, Fagan J, Kennedy E, Conneely M, Crowe B, Lorenz I (2016). "Optimisation of the zinc sulphate turbidity test for the determination of immune status." *The Veterinary record*, **178**, 169. ISSN 2042-7670. `doi:10.1136/vr.103401`.

Hudgens KA, Tyler JW, Besser TE, Krytenberg DS (1996). "Optimizing performance of a qualitative zinc sulfate turbidity test for passive transfer of immunoglobulin G in calves." *American journal of veterinary research*, **57**, 1711–1713. ISSN 0002-9645.

McEwan AD, Fisher EW, Selman IE, Penhale WJ (1970). "A turbidity test for the estimation of immune globulin levels in neonatal calf serum." *Clinica chimica acta; international journal of clinical chemistry*, **27**, 155–163. ISSN 0009-8981.

Bovine Abortion

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Abortion in ruminants is a significant cause of economic loss. Laboratory diagnosis is central to managing and controlling outbreaks, limiting their spread and preventing zoonotic infections. While many pathogens can cause abortion in cattle, no single diagnostic test can be used to identify all aetiologies. Regional Veterinary Laboratories (RVLs) foetal investigations primarily focus on the most likely aetiologies and those with zoonotic potential. Brucellosis, an important disease, has been eradicated in Ireland following a successful statutory program; however, continuous surveillance remains crucial for both public and animal health considerations.
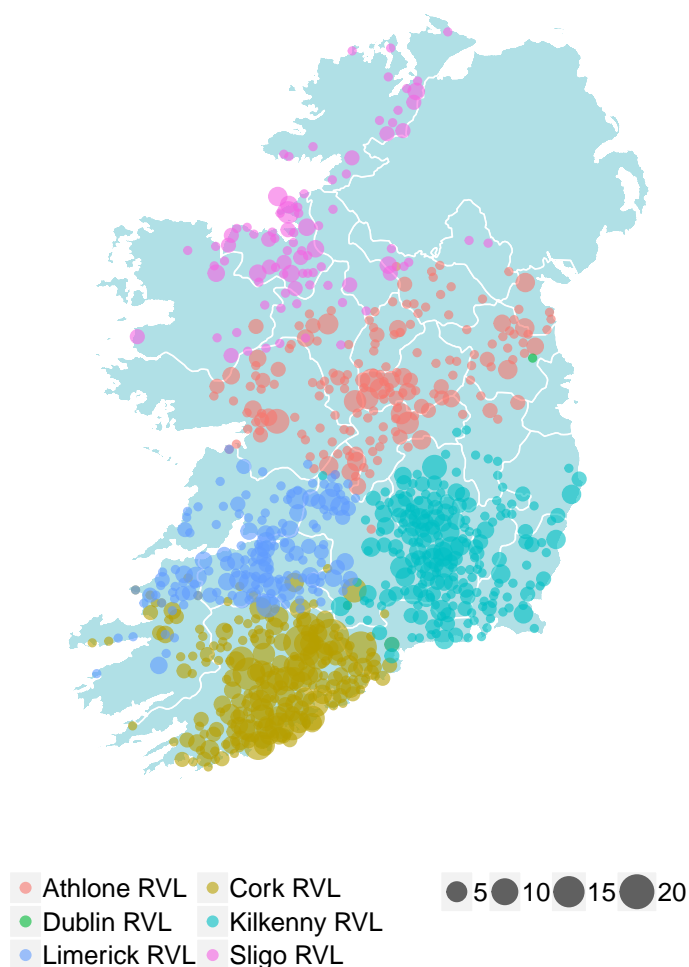
Bovine Abortion

A threshold of 5 *per cent* foetal mortality rate of is recommended when deciding whether to instigate an investigation, although in some instances, a cluster of cases in quick succession may be more critical in deciding to submit aborted material to the laboratory. The aetiology of bovine abortion is broad and diagnostic success rate is low, however, adequate sampling, appropriate laboratory testing, clinical history, vaccination programme and epidemiological information increase chances of reaching an aetiologic diagnosis.

Aborted foetus, placenta and maternal serum constitute the minimum sampling requirements for an abortion investigation. The inclusion of placenta is critical for diagnosis of some mycotic and bacterial abortions where placenta is the primary tissue affected. Submission of blood samples from aborting cows can provide valuable information by either excluding some organisms, i.e. *Neospora caninum,* or reinforcing diagnosis of other agents, as is the case in *Salmonella Dublin* abortions.

In 2018, 1914 bovine foetuses, stillbirths and foetal material (placenta, foetal organs, abomasal contents, etc.) were tested for Brucellosis and routine foetal cultures in RVLs. This section presents some of the most common aetiologies diagnosed in RVLs. Bacterial, fungal and protozoal agents are the most frequent abortifacients detected; they can be divided into primary and secondary pathogens. Primary pathogens can cross an intact placenta and cause placentitis and fetopathy. Secondary pathogens are opportunistic organisms that count on maternal immunosuppression or placental damage to cause abortion (Mee and Sanchez-Miguel, 2015).

Figure 1: Submissions of bovine foetuses and foetal material aggregated and mapped at their DED (District Electoral Division), and colour-coded by Regional Veterinary Laboratory.

In routine RVL foetal culture workflows, most bacteria associated with abortion in cattle can be isolated by aerobic culture from abomasal contents, placenta or foetal organs. Anaerobic culture is not usually carried out in this workflow, therefore, anaerobic bacteria may be underreported as abortifacient. Similarly, organisms

that required specific media culture, e.g. *Mycoplasma spp*, *Ureaplasma* or *Chlamydophila spp*, may also be underreported.

Primary Pathogens

Agents such as *Brucella abortus*, *Salmonella Dublin*, *Leptospira harjo*, *Listeria monocytogenes*, *Aspergillus fumigatus*, *Neospora caninum*, BVDv, BHV-1, etc., are capable of crossing intact placentas causing placentitis, fetopathy and/or luteal regression; they are classified as primary abortifacients.

> Some abortifacients are zoonotic and can pose a serious threat to the health of veterinary practitioners and farmers. It is advisable to always take precautions when handling foetuses or aborted material.

***Salmonella Dublin* abortion.** Salmonella abortions in Ireland are predominantly associated with *Salmonella Dublin* serotype. In 2018, 4.2 *per cent* of bovine abortions were attributed to *Salmonella Dublin* (Table 1). This type of abortion usually occurs in the second half of pregnancy with bacterial translocation from the intestine to the placenta. Typically, aborted foetuses are autolysed, occasionally emphysematous, and smell of *rotten eggs* due to production of hydrogen sulphide. A diagnosis of *Salmonella Dublin* can also be reached with maternal serology. In non-vaccinated aborting cows a single blood sample can be up to 85 *per cent* accurate in predicting a *S. Dublin* foetal culture positive result [@Sanchez-Miguel2018].

Table 1: Number of extitSalmonella Dublin isolates in foetal material in 2018 (n= 1970).

Result	No. of Cases	Percentage
Negative	1887	96
Positive	83	4

Salmonella Dublin abortions have a well documented seasonal distribution in Ireland (DAFM, 2016) characterised by a steady increase towards October/November, as shown in Table 2 and Figure 2; this seasonal distribution emphasises the importance of choosing the right time to vaccinate for *Salmonella Dublin*.

Table 2: Monthly count and percentage of Salmonella culture results in foetal material during 2018 (n= 1970).

Month	Total	Positive	Percentage
Jan	624	16	3
Feb	386	1	0
Mar	136	0	0
Apr	66	0	0
May	39	0	0
Jun	26	0	0
Jul	29	0	0
Aug	35	1	3
Sep	51	4	8
Oct	113	11	10
Nov	200	23	12
Dec	265	27	10

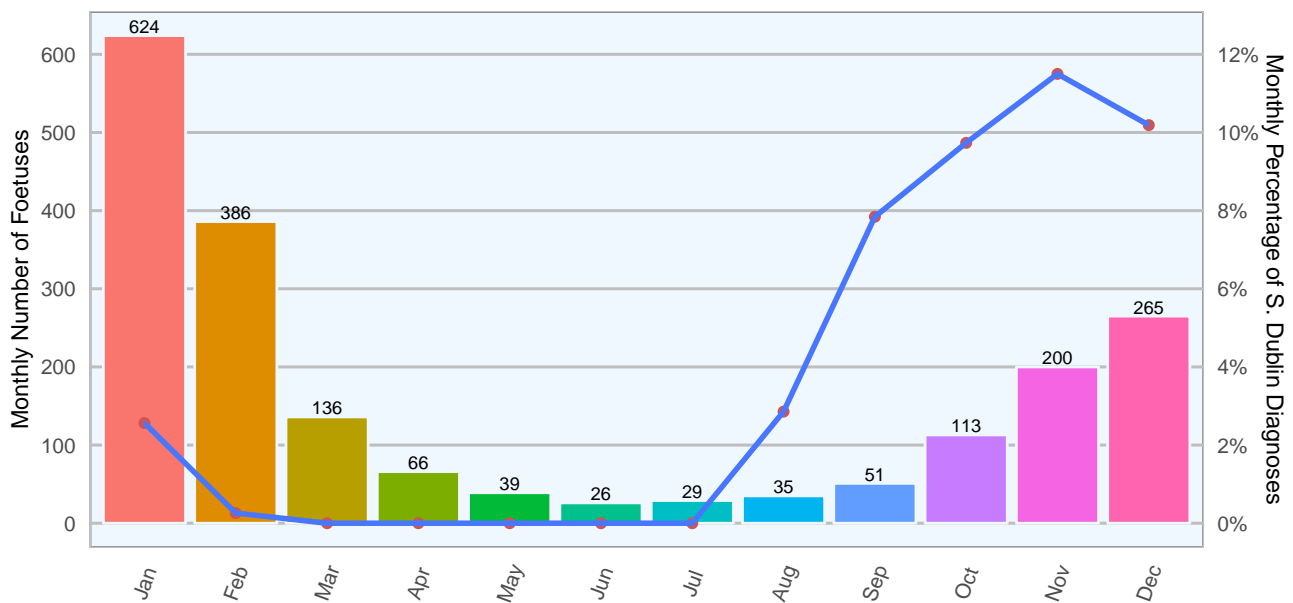**Table 3:** Frequency of detection of other primary abortion pathogens in foetal culture during 2018 (n= 1970)

Organism	No. of cases	Percentage
Trueperella pyogenes	131	6.8
Bacillus licheniformis	101	5.3
Listeria moncytogenes	42	2.2
Aspergillus spp	12	0.6

Nine *Salmonella spp.* serotypes other than *S. Dublin* were also isolated in foetuses, eight were *Salmonella typhimurium* and one *Salmonella Indiana* 4.

Listerial abortion. *Listeria monocytogenes* and possibly *L. ivanovii* may cause sporadic abortions in all stages of pregnancy. *Listeria spp.* are widespread in the environment; clinical disease is associated with ingestion of poorly fermented silage. Following ingestion, *Listeria monocytogenes* proliferates firstly in placenta, then in foetal liver causing septicaemia and, lastly, death.

The proportion of diagnosed abortions attributed to *L. monocytogenes* infection is usually is low, amounting to 42 (2.2 *per cent*) of the total abortions during 2018 (Table 3). Most listerial abortions have a sporadic occurrence and are rarely associated with listerial encephalitis. A markedly autolysed foetus is usually aborted in the third trimester.

Leptospiral abortion. *Leptospira hardjo* has adapted to cattle, which serve as maintenance host. *Leptospira spp.* is labile and difficult to culture, hence diagnosis normally relies on detection of antibody titres by foetal serology or, occasionally, on Fluorescent Antibody Test (FAT) on foetal kidney smears using multivalent antisera or PCR for pathogenic *Leptospira spp.*. Leptospirosis

Figure 2: Annual distribution of foetal submissions (bars) and *Salmonella Dublin* isolates (line) from foetal bacterial cultures as a percentage of monthly bovine submission (n= 1970).

is likely to be underdiagnosed as cause of abortion in cattle due to poor diagnostic tests available at present. This may explain the variability in percentages of diagnosed cases from year to year and laboratory to laboratory. Abortion is frequently the only clinical sign observed in a herd, except in lactating cattle where signs of acute leptospirosis may include agalactia, mastitis, fever, haemolytic anaemia, haemoglobinuria and icterus.

Minor Primary Abortifacients (sporadic abortions). Some bacteria can cause maternal bacteraemia, reach the gravid uterus and foetus and progress to causing sporadic abortion. Amongst them, *Truperella pyogenes*, with 131 (6.8 *per cent*) and *Bacillus licheniformis* with 101 cases (5.3 *per cent*) are listed as the most common agents of sporadic abotion.
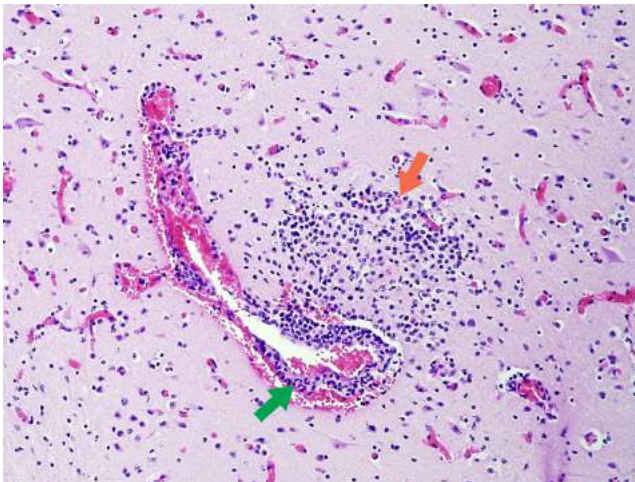
Protoozoal abortion

Since its identification in the 80's, neosporosis, caused by the protozoan *Neospora caninum*, has emerged as one of the most common infectious causes of abortion in cattle worldwide. Acutely infected dogs shed *N. caninum* oocysts in faeces contaminating the environment. Cattle may become infected by ingesting oocysts (from infected aborted material or environment) or by acquiring the parasite in utero. The parasite invades and

multiplies within placental cells causing impairment of oxygen and nutrient transfer from mother to foetus, leading to foetal death. *N. caninum* may also reach foetal organs causing a non-suppurative inflammatory reaction; foetal brain, followed by myocardium, are the preferred sites to detect characteristic lesions (Figure 3).

In 2018, *N. caninum* was detected in 81 foetuses, either by foetal serology, histological examination or by both methods. This figure represents a similar proportion of cases in 2018 compared to 2016, 4 *per cent* and 3.5 *per cent* of the total number of foetuses respectively; however, it is essential to bear in mind that not every submitted foetus is tested for *N. caninum*.

Most *N. caninum* abortions occur in mid to late gestation, but not all cows that are infected with *N. caninum* will abort. Nonetheless, infected cows are more likely to abort than uninfected. *N. caninum* abortions are more frequently seen in heifers or recently infected cows. This type of abortion follows different patterns that are dependent on level of exposure to parasite and predominant route of transmission within the individual herd. These patterns are:

- Epidemic abortions (abortion storms): due to primary infection of naive cows that are exposed to a single source of infection such as ingestion of

SANCHEZ-MIGUEL C.

Figure 3: Protozoal encephalitis: non-suppurative encephalitis (orange arrow), mild vasculitis (green arrow) and congestion, associated with *Neospara caninum* in the brain of a bovine foetus. Photo: Cosme Sánchez-Miguel.

aborted membranes, feed or water contaminated with *N. caninum* oocysts.

- Endemic abortions: chronic abortion episodes spanning several years and found within infected family lines as a result of recurrent transplacental (vertical) transmission.
- Sporadic abortions: occasional occurrence of abortions within a herd

In RVLs, diagnosis of bovine neosporosis at *post-mortem* is based on presence of lesions consistent with protozoal damage in infected tissues (brain, myocardium and placenta) and detection of specific antibodies in the dam or foetal blood or fluids. Detection of *N. caninum* by PCR or immunohistochemistry in tissues is not undertaken in routine foetal submissions and is only carried out occasionally in herd investigations. Diagnostic of *N. caninum* abortions poses a two-fold challenge: tissue lesions, though very distinctive (necrotic foci and mononuclear cell infiltrates) are only suggestive of protozoal abortion and foetal serology depends on quality of the sample (absence of autolysis) and age of the foetus (mature enough to have produced antibodies). In addition to that, a serology positive *N. caninum* test result should be viewed with caution as calves are not always adversely affected by the protozoa and abortion could have been caused by a different abortifacient agent.

Control options for Neospora infection are based on biosecurity, identification of infected animals and appropriate management decisions. An integrated con-

Table 4: Combined frequency of detection of selected abortion agents on routine foetal culture.

Organism	No of Cases
Coliforms	333
Streptococcus spp	61
Bacillus spp	11
Yeasts and Fungi	10
Salmonella spp (other than S. dublin)	9
Staph. spp	9
Listeria spp	7
Mannheimia haemolytica	6
Pseudomonas spp	5
Pasteurella multocida	2
Histophilus somnus	1
Yersinia pseudotuberculosis	1

trol programme should include measures aimed at minimising chances of horizontal (ingestion of infective oocysts) and vertical (from mother to foetus) transmission thus interrupting the parasite life-cycle.

> **Prevent Neospora transmission by enhancing biosecurity**
>
> - Dispose of aborted materials (foetuses and placenta) promptly and safely, as tissues infected by Neospora and other abortificient agents pose a high risk of infection.
>
> - Prevent dogs from having access to cattle areas, especially calving areas.
>
> - Prevent dogs from having access to cattle feed, pastures, fields for production of cattle forage and water sources.
>
> - Control rodents on the farm. Rodents may act as intermediate hosts for Neospora and they may pose a risk if ingested by dogs.

Secondary Pathogens

These organisms form a diverse group of bacteria associated with opportunistic infections of placenta and foetus; they are incapable of transplacental infection unless

munocompromised. Since their presence is widespread in the environment, they can potentially cause maternal bacteraemia, reach the gravid uterus and trigger an opportunistic abortion. Table 4 summarises the number of cases in 2018, amongst them *Streptococcus spp.*

(61 cases isolated), *Bacillus spp* (61), *Staphylococcus spp.* (9), *Mannheimia haemolytica* (6), *Pseudomonas spp.* (5), *Pasteurella multocida* (2), *Histophilus somnus* (1), *Yersinia pseudotuberculosis* (1).

Their presence in tissues of aborted foetuses should not be considered as definitive evidence of cause of abortion. For secondary pathogens to be regarded as the cause of abortion, they must be isolated from foetal material, have produced representative lesions and primary pathogens must have been excluded. Secondary pathogens usually cause sporadic abortions; multiple abortions can be a consequence of maternal health issues that facilitate haematogenous infections.

Figure 4: Angioinvasive: *Aspergillus fumigatus* invading through the endothelial cells of a blood vessel. Grocott's methenamine silver stain. Photo: Cosme Sánchez-Miguel.

Mycotic abortions. Mycotic abortions usually occur in the third trimester of pregnancy. *Aspergillus spp.* (Figure 4) and *Mucor spp.* are the most common organisms isolated (10 cases in 2018). Clinical signs in dams, apart from placental retention, are infrequently observed. Diagnosis of fungal abortion is based on demonstration of fungi and presence of consistent gross and histopathological lesions. Grossly visible placental lesions include a leathery, diffusely thickened intercotyledonary membrane with necrotic haemorrhagic infarcts in cotyledons. Foetal lesions may be absent and autolysis minimal. Occasionally, locally extensive circular skin lesions may be present on foetuses. Microscopically, there is a severe suppurative placental vasculitis with intralesional fungi (Schlafer and Miller, 2007). Inflammatory lesions associated with fungal invasion may be present in foetal respiratory and digestive systems. Direct identification of fungi using a potassium hydroxide wet-mount examination of lesion scrapings may facilitate diagnosis.

References

DAFM (2016). "All-island Animal Disease Surveillance Report." URL https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/labservice/rvlreports/AIDSRReport016230118.pdf.

Mee J, Sanchez-Miguel C (2015). "Abortion investigations – can practitioners do better?" *Proc. CAVI Conf., Ireland*, pp. 95–101.

Schlafer D, Miller R (2007). *Palmer's Pathology of Domestic Animals*. Saunders Elsevier, Philadelphia.

Bovine Mastitis

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Dairy farmers are committed to producing food of the highest standard for consumers. Cellcheck, the National Mastitis Control Programme coordinated by Animal Health Ireland, in association with farmers, milk processors, service providers and Government, have been focusing on reducing somatic cell counts of milk produced in Ireland. Farmers are being encouraged to collect and submit milk samples from cows with cases of clinical and suspect subclinical mastitis for culture and antimicrobial susceptibility testing. Bacteria are responsible for virtually all cases of mastitis; by identifying the agent responsible, important information about possible source of infection may be gathered, enabling a focus for control measures to improve milk quality.

Figure 2: *Staphylococcus aureus* growing on a blood agar plate. Photo: Alan Johnson.

Milk Culture in RVLs

All RVLs follow a similar procedure for culturing milk samples. Samples are initially tested for inhibitory substances, such as antibiotics, which can interfere with bacterial growth in the laboratory. At least four different types of agar plates are used to culture each milk sample. Using a sterile swab, a small quantity of milk is spread on to each plate (Figure 1). These plates are incubated at 37 °C for 24 *hours*. If, on inspection, no bacterial growth has occurred at that stage, plates are incubated for a further 24 *hours*. If there is no change after 48 *hours* of culture, the result *no significant growth* is entered.

Figure 1: Inoculation of agar plates for culture of a milk sample in Limerick RVL. Photo: Alan Johnson.

If bacterial growth is seen on plates, further tests are carried out to identify organisms growing (Figure 2). If milk sample has been contaminated, cultures usually yield a mixed bacterial growth (Figure 3); in these cases it can be difficult to identify the significant bacterial species and the result is entered as *mixed bacterial growth*. Contamination usually occurs when bacteria from sources other than milk inside the udder enter the sample. This could be from skin of the udder, sampler's hands or from inside of the container itself, if the latter is not sterile.

For additonal information, see the Animal Health Ireland webpage.

Table 1: Relative frequency of mastitis isolates in milk samples submitted to RVLs in 2018 (n= 3413).

Result	No. of cases	Percentage
Staphylococcus aureus	911	**26.7**
No Significant Growth	591	**17.3**
Other Isolates	578	**16.9**
Contaminated	557	**16.3**
Streptococcus uberis	407	**11.9**
E. coli	193	**5.7**
Streptococcus dysgalactiae	109	**3.2**
Trueperella pyogenes	41	**1.2**
Bacillus spp.	26	**0.8**

RVLs have seen an increase in the number of samples received for culture and antimicrobial sensitivity testing. In 2018, 3413 individual milk samples were

Figure 3: Mixed bacterial growth on a blood agar plate following culture of a contaminated milk sample. Photo: Alan Johnson.

received for testing (Table 1).

Staphylococcus aureus. This organism continued to be the most commonly pathogen isolated in cases of mastitis by RVLs in 2018 (Figure 2). It was identified in 911, 26.69 *per cent*, of samples submitted (Table 1). *Staph. aureus* is the main cause of contagious mastitis and typically, though not always, spreads from cow-to-cow by contact with infected milk on cluster liners or on milker's hands. It can be difficult to cure, particularly during lactation and culling is frequently the best option in older infected cows with persistently high somatic cell counts.

Other isolates, mostly coagulase-negative *Staphylococcus spp.*, were cultured from 578, 16.94 *per cent*, milk samples.

Staphylococcus aureus mastitis

Herds with mastitis caused by *Staphylococcus aureus* infection should reassess their milking hygene. To prevent spread of infection, infected cows should be segregated and milked last or in a separate unit.

Streptococcus uberis. This organism is described as an environmental mastitis pathogen. It is usually due to faecal contamination of surfaces. Sub-optimal housing and poor udder hygiene can increase the risk of infection (Barrett *et al.*, 2005). In addition, *Strep. uberis* also has some characteristics of a contagious pathogen and can be spread from cow-to-cow at milking time. *Strep. uberis* was isolated from 407, 11.92 *per cent*, of milk samples cultured during 2018 (Table 1).

Truperella pyogenes. This is the most commonly isolated pathogen in cases of summer mastitis. It is associated with a suppurative foul-smelling secretion and loss of the quarter for milk production. Insect vectors are considered central to its spread, hence its association with the summer months.

A similar syndrome can be found during the indoor (in-house) season following a teat injury.

T. pyogenes was isolated from 41, 1.2 *per cent*, milk submissions.

Contaminated samples. In 2018 the number of samples described as contaminated was high at 557, 16.32 *per cent*. When collecting milk samples for culture, it is very important to collect them in a sterile manner. This will ensure that any bacteria isolated has originated from within the udder, and not from the teat skin, milker's hands or non-sterile equipment. Contaminated samples usually result in growth of coliforms or a mixture of bacterial species

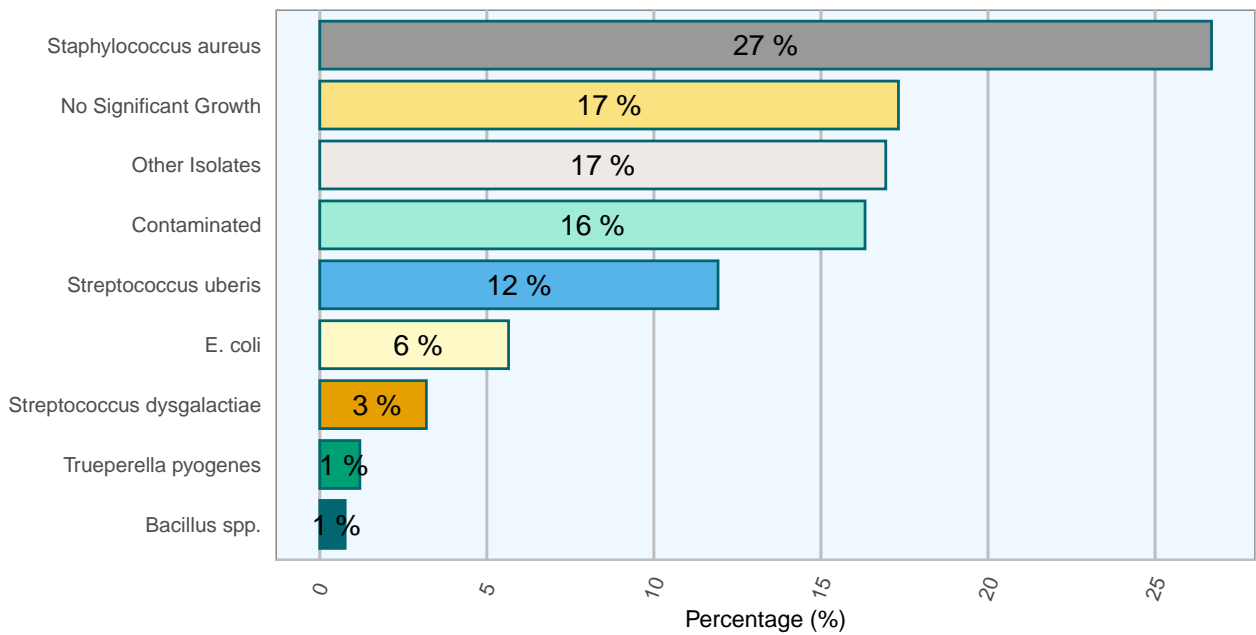**Table 2:** Number of milk samples submitted to the RVLs from 2010 to 2017.

2010	2011	2012	2013	2014	2015	2016	2017
5355	3469	2899	3329	2288	2315	2849	2416

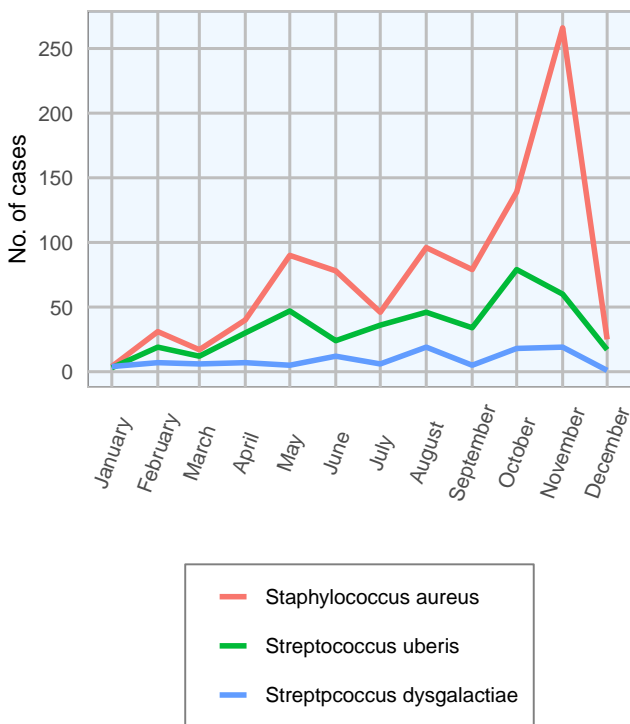### Milk Sample Collection for Bacteriology: **Materials for Sampling**

The quality of milk samples taken for laboratory examination is extremely important. An aseptic technique for sample collection is a necessity. Contaminated samples lead to misdiagnosis, confusion and frustration. If dry cow therapy decisions are to be based on the results of a small number of milk samples, it is vital that proper procedures are followed. Materials for sampling:

- Disposable latex gloves.
- Sterile screw-top plastic tubes (20 ml capacity approximately).
- Cotton wool balls soaked in 70 % alcohol or medicated wipes.
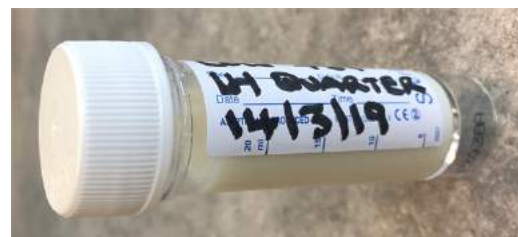- Paper towels.

Over the last number of years there has been a fall in isolation rates for *Staphylococcus aureus* and a rise in

Figure 4: Relative frequency of selected mastitis pathogens during 2018.

Figure 5: Monthly isolation counts of important mastitis pathogens from milk submissions in 2018 (n= 3413).

isolation rates for *Streptococcus uberis* (Figure 6). This may be associated with increased attention by farmers to controlling the spread of infections from cow to cow at milking time.

Figure 7: Labeled milk sample bottle with date and quarter sampled. Photo: Alan Johnson.

However, results from 2018 show a reverse in this trend with a rise in isolation of *Staph. aureus* and a drop in *Strep. uberis*. Reasons for this are not clear but may be due to weather conditions during 2018; a cold winter and spring followed by a summer drought.

In many countries, *Streptococcus uberis* is the most commonly isolated mastitis pathogen.

Mastitis–associated organisms isolated in milk (2010–2017)

Based on bovine milk samples submitted to the RVLs

| 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 |

Staph. aureus — 36.5   39.2   36.4   37.3   38.2   31.7   26.5   23.5 — Staph. aureus

15 — Strep. uberis

11.7

Strep. uberis — 6.03   7.9   8.45   9.19   8.92   9.16

Strep dysgalactiae 4.8   3.43   3.41   4.27   3.54   3.28   2.84   3.52 rep dysgalactiae

0.62   0.57   1.05   1.25   1.23   1.16 — T. pyogenes

Annual percentages of major pathogens isolated in mastitis culture

Figure 6: Mastitis-associated Organisms Isolated in Milk (2010-2017).

Milk Sample Collection for Bacteriology: **Sampling Technique**

1. Take the sample before milking and before any treatment is given.
2. Label the tubes prior to sampling with name/creamery number/herd number, cow number, quarter and date.
3. Using a hand or paper towel brush any loose dirt, straw or hair from teat or underside of the udder. Washing should be avoided if possible. However, if teat is soiled it should be washed and carefully dried with paper towels.
4. Put on gloves.
5. Soak a number of cotton wool balls in alcohol.
6. Clean teat thoroughly with alcohol soaked cotton wool or the medicated wipes until it is thoroughly clean.
7. Remove cap from sampling tube. Place cap on a clean surface with closing side up. Hold open tube at an angle of 45 degrees (holding it straight up will allow dust etc. to fall inside). Using your other hand, discard first few streams of milk on to the ground before collecting three or four streams in the tube.
8. Replace cap on sampling tube (Figure 7).
9. If you feel that some contamination has occurred, discard sample and use a new tube.
10. Place labelled tube in a fridge and cool to 4 °C. This is very important.
11. Sample should be taken to the laboratory as quickly as possible. If sample is handed to milk tank driver for delivery, ensure that it is placed in a cool box.
12. If sample is not going to a laboratory immediately, it must be refrigerated until delivery.

References

Animal Health Ireland (2019). "Farm Guidelines for Mastitis Control." URL http://animalhealthireland.ie/?page_id=52.

Barrett DJ, Healy AM, Leonard FC, Doherty ML (2005). "Prevalence of pathogens causing subclinical mastitis in 15 dairy herds in the Republic of Ireland." *Irish Veterinary Journal*, **58**(6), 333. ISSN 2046-

0481. doi:10.1186/2046-0481-58-6-333. URL https://doi.org/10.1186/2046-0481-58-6-333.

Bovine Respiratory Disease

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Bovine respiratory disease (BRD) remains an important cause of morbidity and mortality in cattle. Good management practices, early detection of disease and appropriate treatment of infection are critical to avoid, minimise and control disease outbreaks. Clinical signs consistent with BRD may include but are not restricted to: depression, pyrexia, anorexia and loss of condition, serous to muco-prurulent ocular and/or nasal discharges with/without diphtheritic plaques, increased respiratory rate and effort with a variable abdominal component, stridor breathing and/or the presence of a cough, abnormal lung sounds ranging from an absence to the presence of crackles and wheezes, increased heart rate and abortion.

Following exposure, disease outcome depends on a range of pathogen, host and environmental effects. To cause BRD, pathogens must successfully manipulate or evade host defences, including the resident microflora, mucocillary escalator, antimicrobial peptides and proteins, and innate and adaptive immune responses (Ackermann *et al.*, 2010; Caswell, 2014).

Bacterial Bovine Respiratory Disease

RVL submissions are an example of a passive surveillance system. In 2018, 542 submitted carcasses were diagnosed as BRD on post mortem examination. A breakdown of the number of cases, by agent and age group, can be seen in Table 1. Where two or more organisms may have been identified, the final diagnosis represents what would have been considered by the pathologist as the primary cause of disease.

Throughout 2018, bacterial agents were identified as the main cause of BRD in 64.3 *per cent* of submissions (Tables 1, 2 and 3). Reflecting the multi-factorial nature of the BRD complex, bacterial pathogens may also be identified in healthy cattle, but at a lower rate than those with acute disease (Timsit *et al.*, 2017).

Mannheimia haemolytica and *Pasturella multocida* are Gram-negative commensals of the nasopharynx and an important cause of respiratory disease in cattle, sheep and goats. In particular, causing the cattle disease known as shipping fever or bovine pneumonic pasteurellosis/ mannheimiosis. Healthy animals can carry *M. haemolytica* as a commensal without developing clinical signs. When animals are stressed (e.g. at housing or during transportation), and/or become infected with viruses. When animals are stressed, for example

Figure 1: Characteristic cranioventral fibrinous bronchopneumonia (arrows) caused by *Mannheimia haemolytica*. Photo: Cosme Sánchez-Miguel.

Table 1: Number of cases and percentage (%) by age of the general pathogenic groups detected in the BRD cases diagnosed on post mortem examination (n= 542).

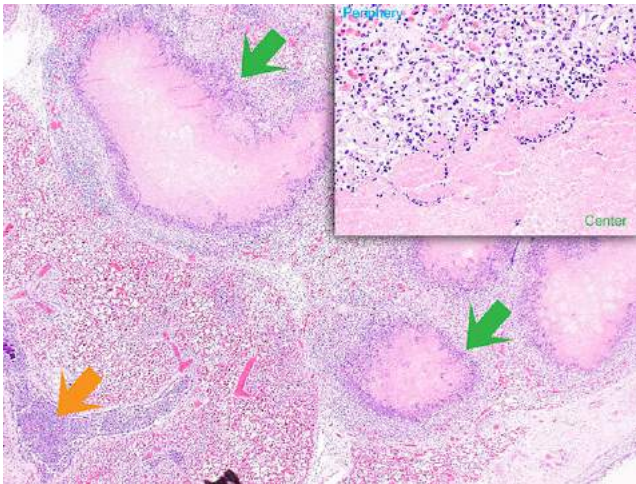| Organism Group	Calves	Weanlings	Adult Cattle	Total
Bacterial	227 (69.2)	76 (55.5)	41 (54.7)	**344 (63.7)**
Viral	38 (11.6)	35 (25.5)	13 (17.3)	**86 (15.9)**
No agent identified	40 (12.2)	5 (3.6)	9 (12.0)	**54 (10.0)**
Parasitic	20 (6.1)	20 (14.6)	10 (13.3)	**50 (9.3)**
Other	1 (0.3)	1 (0.7)	2 (2.7)	**4 (0.7)**
Fungal	2 (0.6)	0 (0.0)	0 (0.0)	**2 (0.4)**

Note:
Calves:1-5 months old,
Weanlings: 6-12 months old
Adult Cattle: over 12 months old

at housing or during transportation, *M. Haemolytica* can replicate and be inhaled into the lower respiratory tract. Other opportunistic pathogens can commonly invade following damage to tissue.

During 2018, the most frequently detected primary pathogens associated with BRD were *P. multocida*, *M. haemolytica* (Figure 1) and *M. bovis*, which accounted for nearly half of all agents diagnosed on *post mortem* examination (see Table 2 and Figure 3). This is consistent with data from the immediately preceding years, with the exception of *M. bovis* which has increased (Figure 8).

Mycoplasma bovis is associated with a characteristic

Figure 2: Bovine lung. Rounded foci of caseous necrosis (green arrow) surrounded by a rim of granulation tissue and centered around the airways in a calf with *Mycoplasma bovis*. Photo: Cosme Sánchez-Miguel.

cuffing bronchopneumonia and, in severe cases, multifocal pulmonary abscessation (Figure 2); it can also cause arthritis and otitis media. *Mycoplasma bovis* associated pneumonia can occur at any age. It has been associated with outbreaks in feedlot cattle and sometimes is followed by an outbreak of polyarthritis following the initial respiratory presentation. *Mycoplasma bovis* is capable of causing pneumonia on its own, or as part of the BRD complex where viral infections often cause the initial insult that damages the respiratory mucosa. This can reduce the activity of the cilia and weaken the immune defences of the respiratory tract. The animal's immune status is important in the development of mycoplasma pneumonia; failure of passive transfer is a risk for the increased severity of respiratory disease in young calves.

Similar to the rest of the pneumonia pathogens, non-specific respiratory defences can be compromised by many risk factors such as viral pathogens, changes in environmental temperature, heat or cold stress, overcrowding, transport, poor air quality and poor nutrition. *Mycoplasma bovis* is capable of persisting with or without causing clinical disease for variable periods of time making shedding patterns difficult to predict (Carty, 2017).

Ante-Mortem samples

When choosing what *ante mortem* samples to collect, the suspect pathogen, stage of disease and test to be employed are important considerations:

- BRD associated pathogens may be identified through bacterial culture, viral isolation or molecular techniques such as polymerase chain reaction (PCR) based assays.

- In addition to nasal swabs and blood samples, transtracheal washes and bronchoalveolar lavages may be used for agent identification.

- Samples should be collected from a representative population. Samples from untreated acute clinical cases are ideal for successfully identifying the primary cause of disease, particularly in the case of viruses. Chronic cases often have superimposed secondary bacterial infections. (Cooper and Brodersen, 2010).

In 2018, other less commonly encountered organisms included *Histophilus somni* and *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*) (Table 2). *Histophilus somni* (formerly *Haemophilus somnus*), causes septicemic infection with clinical presentations, including pneumonia, polyarthritis, myocarditis, abortion and meningoencephalitis. The respiratory system is usually the initial site of replication followed by spread to the CNS via the circulation. The CNS form is called thrombotic meningoencephalitis (TEME), previously called TEME. All age group of animals can be infected with *H. somni*, but 6 *months* to 2 *years* tends to be most frequent age of animals affected. Clinical signs include depression, high temperatures, dyspnoea, discharge from eyes and nose and some animals can display stiffness. When *H. somni* is involved in pneumonia it is often overgrown by *Pasteurella spp.* organisms. *H. somni* is an opportunistic pathogen that complicates viral infection and increases the severity of infection with other bacterial agents.
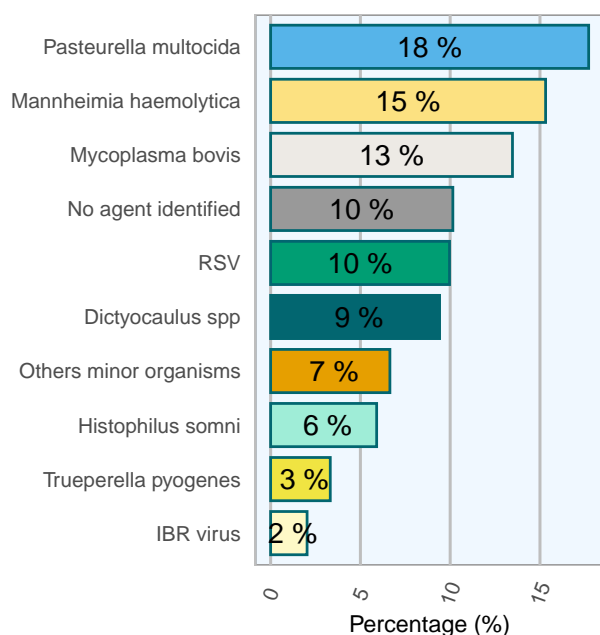
Trueperella pyogenes is an opportunistic bacterium related to various pyogenic infections in animals. A great variety of clinical manifestations has been attributed to *T. pyogenes* infections in domestic animals, includ-

Table 2: Number of cases and relative frequency of the top ten pathogenic agents detected in BRD cases diagnosed on post-mortem examination, (n= 542).

Organism	No. of cases	Percentage
Pasteurella multocida	96	**17.7**
Mannheimia haemolytica	83	**15.3**
Mycoplasma bovis	73	**13.5**
No agent identified	55	**10.1**
RSV	54	**10.0**
Dictyocaulus spp	51	**9.4**
Others minor organisms	36	**6.6**
Histophilus somni	32	**5.9**
Trueperella pyogenes	18	**3.3**
IBR virus	11	**2.0**

Table 3: Count and percentage by age goup of the general specific organisms detected in BRD on post mortem examination, (n= 542)

Organism Group	Calves	Weanlings	Adult Cattle
BHV4	2 (0.6)	0 (0.0)	4 (5.3)
Bibersteinia trehalosi	1 (0.3)	1 (0.7)	2 (2.7)
BVD virus	2 (0.6)	0 (0.0)	0 (0.0)
Coronavirus	0 (0.0)	2 (1.5)	0 (0.0)
Dictyocaulus spp	20 (6.1)	20 (14.6)	10 (13.3)
Fungal	1 (0.3)	0 (0.0)	0 (0.0)
Histophilus somni	22 (6.7)	6 (4.4)	4 (5.3)
IBR virus	4 (1.2)	1 (0.7)	6 (8.0)
Mannheimia haemolytica	61 (18.6)	12 (8.8)	10 (13.3)
Mycobacterium bovis	2 (0.6)	0 (0.0)	1 (1.3)
Mycoplasma bovis	48 (14.6)	15 (10.9)	10 (13.3)
No agent identified	40 (12.2)	5 (3.6)	9 (12.0)
Other	1 (0.3)	1 (0.7)	2 (2.7)
Others minor organisms	24 (7.3)	9 (6.6)	3 (4.0)
Pasteurella multocida	51 (15.5)	36 (26.3)	9 (12.0)
Pasteurella spp	0 (0.0)	1 (0.7)	1 (1.3)
PI3	1 (0.3)	3 (2.2)	0 (0.0)
RSV	28 (8.5)	24 (17.5)	2 (2.7)
Salmonella dublin	5 (1.5)	0 (0.0)	0 (0.0)
Trueperella pyogenes	15 (4.6)	1 (0.7)	2 (2.7)

Note:
Calves:1-5 months old,
Weanlings: 6-12 months old
Adult Cattle: over 12 months old

ing mastitis, pneumonia and metritis. Usually, it is a secondary pathogen in pneumonia where tissues have been previously acutely damaged by other pathogenic respiratory agents.

Figure 3: Relative frequency of the top ten pathogenic agents detected in BRD cases diagnosed on post-mortem examination, (n= 542).

Ante-Mortem samples (continued)

- Nasal swabs target upper respiratory tract pathogens (Cooper and Brodersen, 2010) and help identify the presence of respiratory viruses (Caswell *et al.*, 2012). Multiple nasal swabs from the same animal or multiple animals can be submitted and pooled for PCR analysis.

- Transtracheal washes and bronchoalveolar lavages are optimal for detecting lower respiratory tract pathogens and are preferable to identify the presence of BRD associated bacteria; they also facilitate cytological examination (Cooper and Brodersen, 2010; Caswell *et al.*, 2012).

- The humoral immune response can be identified through serological techniques such as enzyme linked immunosorbant assays (ELISAs) using blood samples; the presence of antibodies may indicate exposure, previous vaccination or passive immunity.
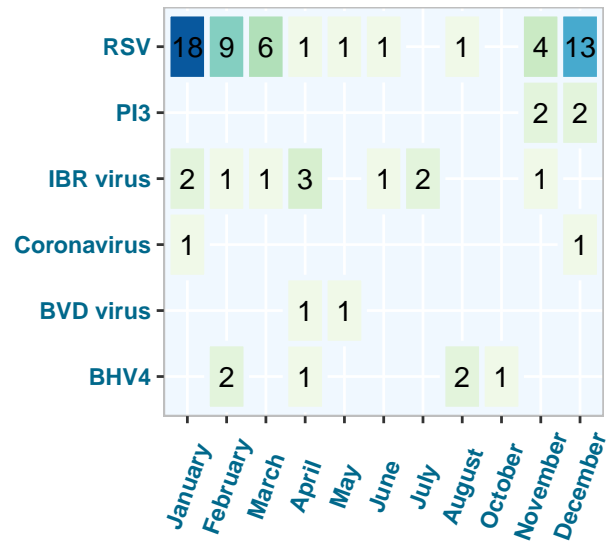
Bibersteinia trehalosi (previously known as *Pasteurella trehalosi*) is a commensal organism of upper gastrointestinal tract. It is thought that under stressful conditions the bacteria can multiply rapidly and spread to the lungs and other organs, causing an acute systemic infection. *Bibersteinia trehalosi* is an important pathogen of sheep, typically associated with acute systemic infections causing death in growing lambs. *B trehalosi* is comparatively infrequently identified as a pathogen in cattle; however, isolates are typically associated with bronchopneumonia.

Salmonella Dublin was detected as the causative pathogen in five calves diagnosed with respiratory disease. Enteric, septicaemic, and reproductive diseases are all possible manifestations of Salmonella infection, with pneumonia being a common manifestation of *Salmonella Dublin* infection in calves

Viral Bovine Respiratory Disease

Viral agents were implicated as the primary cause of 15.3 *per cent* BRD cases diagnosed on post mortem examination during 2018 (Table 1). A range of viruses are involved in the BRD complex and may lead to the development of broncho-interstitial pneumonia. As they are inhaled, gross lung lesions typically follow a cranio-ventral distribution and can vary from mild to severe. Often, two or more viruses are present simultaneously. Importantly, viral infection may in turn predispose to bacterial infection. Similarly to the period 2010 to 2017, during 2018 bovine respiratory syncytial virus (BRSV) and bovine herpesvirus-1 (BHV1) were counted among the most frequently identified pathogenic agents, found in 10 and 2 *per cent* of all of BRD cases diagnosed on post mortem examination, respectively (Table 2).

BRSV infections are associated with respiratory disease in young animals. Although capable of independently producing primary respiratory disease, it is an important component of the BRD complex affecting cattle younger than one *year* and ocasionally adults by predisposing animals to secondary bacterial infections (i.e. *M. haemolytica*). Initial exposure to BRSV can produce acute pneumonia, with subsequent exposure usually resulting in milder disease. The spectrum of clinical signs can range from mild to life-threatening in susceptible cattle. In outbreaks, morbidity tends to be high, and the case fatality rate can be 0–20 *per cent*. Fever, dyspnoea, anorexia and depression are typical clinical signs. Gross lesions can include a diffuse in-

Figure 4: Viral respiratory infections in carcasses. Monthly number of viral pneumonia diagnoses by primary microorganism.

terstitial pneumonia with subpleural and interstitial emphysema along with interstitial oedema.

BHV1 can be divided into three subtypes by restriction endonuclease analysis; whereas subtype 1.1 and 1.2a are associated with the development of infectious bovine rhinotraceitis (IBR; Figure 5), subtype 1.2b is associated with infectious pustular vulvo-vaginitis and balano-posthitis (Raaperi *et al.*, 2014). As implicated in respiratory disease, parainfluenza 3 (PI3), bovine herpesvirus 4 (BHV4), bovine viral diarrhoea (BVD) and bovine coronavirus (BoCo) virus were found sporadically but in very low numbers (Table 3).

Figure 5: The trachea (opened) of a bovine with severe suppurative tracheitis caused by Bovine Herpes Virus (IBR-BHV1). Photo: Cosme Sánchez-Miguel.
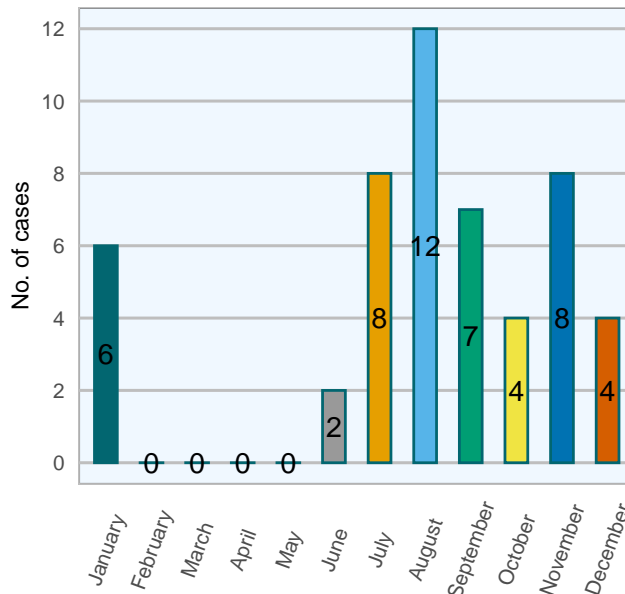
Sampling for virology

Nasal Swabs .
- Select animals for swabbing carefully, for example, those early in the disease which may only present with raised temperature or comrades which have not yet developed clinical signs.
- Use plain swabs, moistered with bottle water or saline solution.
- Swad as deep as posible but at least 1.5 *inches*, expecting resistance.
- Rotate and rub against nasal passage, targeting clear and/or milky secretions if present.
- Multiple swabs (e.g. 5–6) from suitable animals increase chances of detecting the virus, as sheeding can drastically decrease afte 5 *days*.
- Multiple swabs (e.g. 5–6) can be pooled for laboratory testing, minimizing cost while maximazing diagnostic potential.
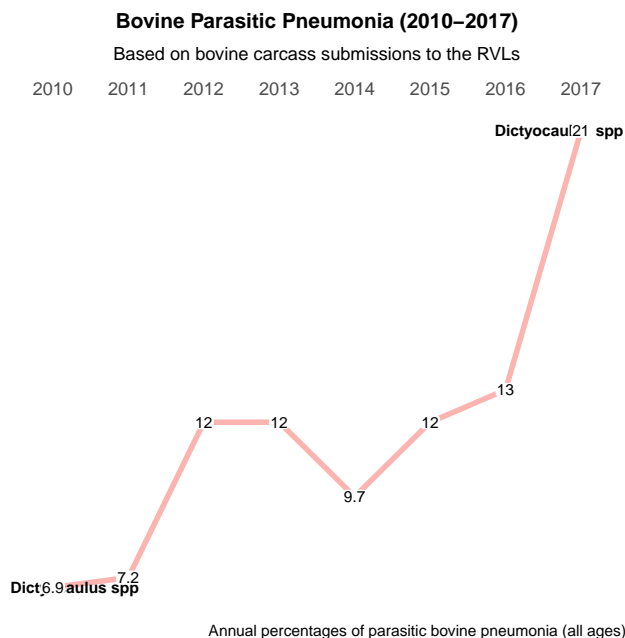
Incidence Trends of BRD

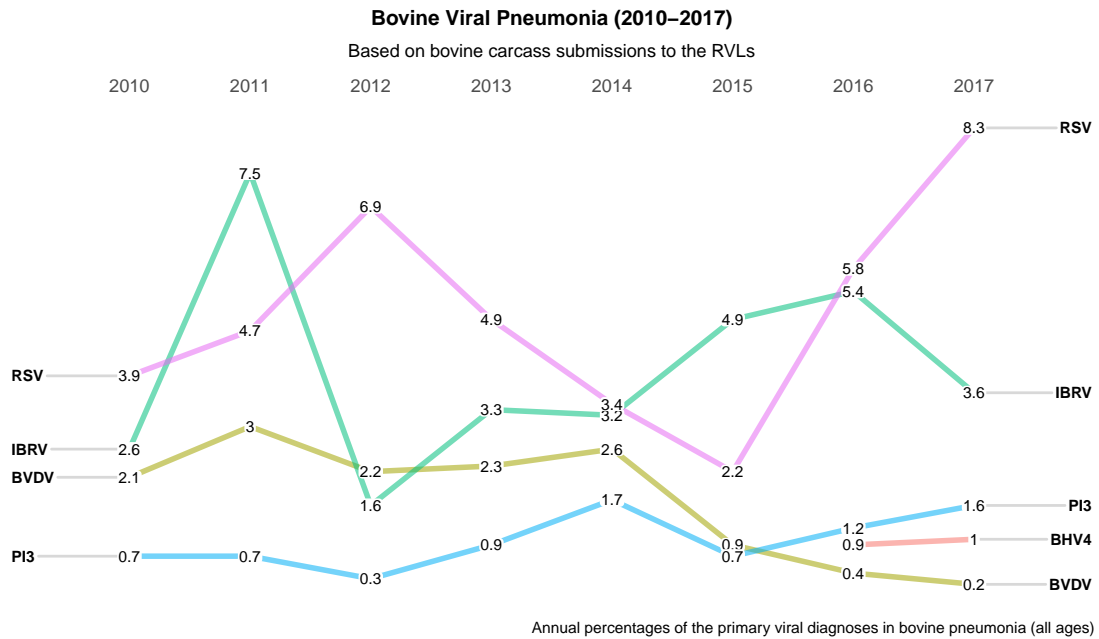### Parasitic Bovine Respiratory Disease

From 2010 to 2017, the RVLs observed an increase in cases of parasitic bronchopneumonia peaking at 21 *per cent* of diagnosed BRD submissions in 2017 (Figure 8). During 2018, *Dictyocaulus* species were detected in 9.4 *per cent* of BRD submissions (Table 1). Lungworm infections are associated with cattle that are or
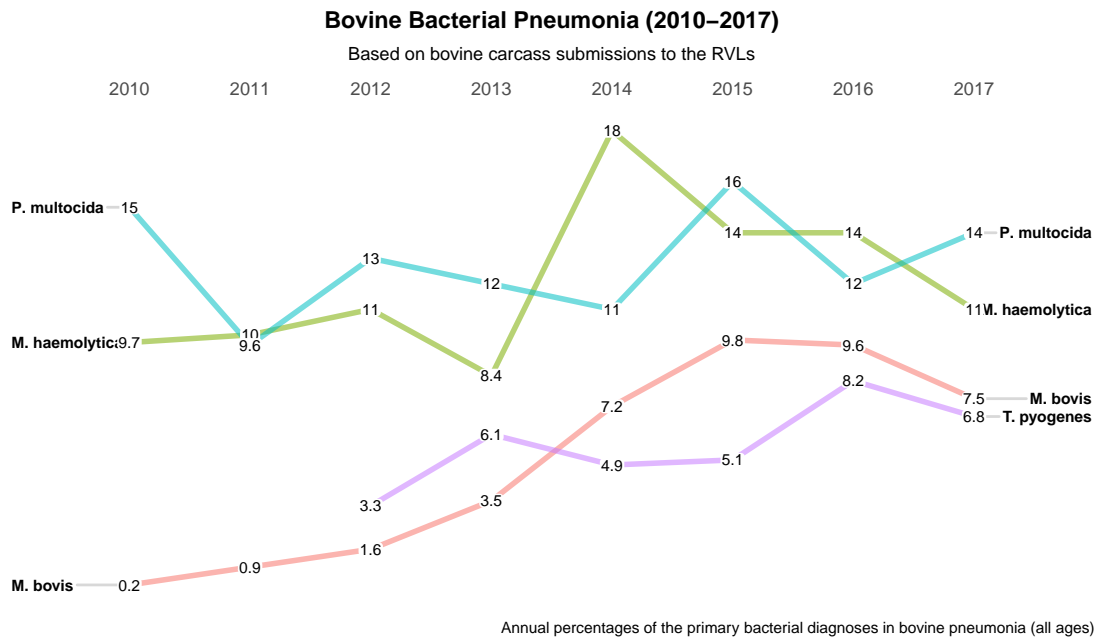

Figure 6: Number of diagnoses of parasitic bronchopneumonia by month during 2018 (n= 51).

Bovine Parasitic Pneumonia (2010–2017)

Based on bovine carcass submissions to the RVLs

Annual percentages of parasitic bovine pneumonia (all ages)

Figure 7: Trends in the incidence of parasitic pneumonia in the carcasses (all ages) submitted to the RVLs from 2010 to 2017.

Bovine Viral Pneumonia (2010–2017)

Based on bovine carcass submissions to the RVLs

Annual percentages of the primary viral diagnoses in bovine pneumonia (all ages)

Figure 8: Trends in the incidence of viral pneumonia in the carcasses (all ages) submitted to the RVLs from 2010 to 2017.

Bovine Bacterial Pneumonia (2010–2017)

Based on bovine carcass submissions to the RVLs

Annual percentages of the primary bacterial diagnoses in bovine pneumonia (all ages)

Figure 9: Trends in the incidence of bacterial pneumonia in the carcasses (all ages) submitted to the RVLs from 2010 to 2017.

Figure 10: Adult *Dictyocaulus viviparus* in a bovine trachea. Photo: Cosme Sánchez-Miguel.

have been recently at pasture and tend to occur in the late summer and autumn. Grossly, adult lungworms are usually found in the caudal bronchi (Caswell *et al.*, 2012). However, animals in the acute prepatent stage can be difficult to diagnose as the adult lungworms will not be visible in the lungs and their eggs will not be found upon analysis of faecal samples (Caswell *et al.*, 2012). Re-infection syndrome may occur in previously infected cattle which are partially immune to the parasites. It typically follows a subsequent challenge from heavily contaminated pasture, leading to the development of respiratory signs. However, the infection will not become patent; adult lungworms will not be found

in the lungs and eggs will not be produced. Following six cases in January 2018, parasitic bronchopneumonia was not subsequently identified until June (Figure 4). Thereafter, it was diagnosed every month, with the largest numbers identified in August.

References

Ackermann MR, Derscheid R, Roth JA (2010). "Innate immunology of bovine respiratory disease." *The Veterinary clinics of North America. Food animal practice*, **26**, 215–228. ISSN 1558-4240. doi:10.1016/j.cvfa.2010.03.001. URL https://www.vetfood.theclinics.com/article/S0749-0720(10)00002-2/abstract.

Carty C (2017). "Mycoplasma bovis." *Veterinary Ireland Journal.* URL http://www.veterinaryirelandjournal.com/images/pdf/large/la_jun_2017.pdf.

Caswell J (2014). "Failure of respiratory defenses in the pathogenesis of bacterial pneumonia of cattle." *Veterinary Pathology*, **51**(2), 393–409. doi:https://doi.org/10.1177/0300985813502821. URL https://journals.sagepub.com/doi/full/10.1177/0300985813502821?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed.

Caswell JL, Hewson J, Slavić u, DeLay J, Bateman K (2012). "Laboratory and postmortem diagnosis of bovine respiratory disease." *The Veterinary clinics of North America. Food animal practice*, **28**, 419–441. ISSN 1558-4240. doi:10.1016/j.cvfa.2012.07.004. URL https://www.vetfood.theclinics.com/article/S0749-0720(12)00055-2/abstract.

Cooper VL, Brodersen BW (2010). "Respiratory disease diagnostics of cattle." *The Veterinary clinics of North America. Food animal practice*, **26**, 409–416. ISSN 1558-4240. doi:10.1016/j.cvfa.2010.04.009. URL https://www.vetfood.theclinics.com/article/S0749-0720(10)00015-0/abstract.

Raaperi K, Orro T, Viltrop A (2014). "Epidemiology and control of bovine herpesvirus 1 infection in Europe." *Veterinary journal (London, England : 1997)*, **201**, 249–256. ISSN 1532-2971. doi:10.1016/j.tvjl.2014.05.040.

Timsit E, Hallewell J, Booker C, Tison N, Amat S, Alexander TW (2017). "Prevalence and antimicrobial susceptibility of Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni isolated from the lower respiratory tract of healthy feedlot cattle and those diagnosed with bovine respiratory disease." *Veterinary microbiology*, **208**, 118–125.

Antimicrobial Resistance

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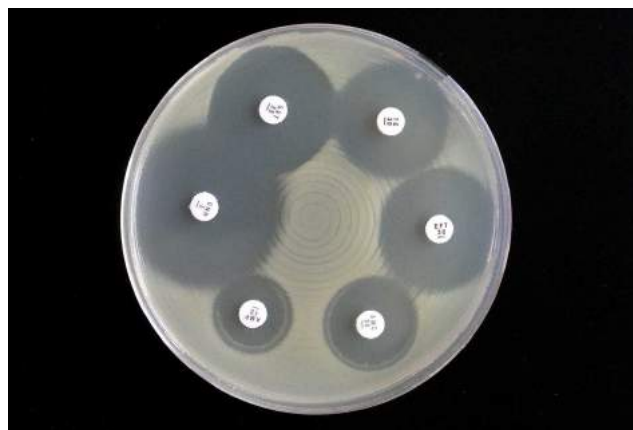
Antimicrobial Resistance (AMR) is defined by the Office International des Epizooties (OIE) as the emergence of resistance amongst bacteria, viruses, fungi and parasites to products known as antimicrobials that are designed to treat them. The OIE has also stated that 'the overuse and misuse of antimicrobials in human, animal and plant sectors has dramatically accelerated the emergence of AMR. Consequently, minimising the emergence and spread of AMR requires a co-ordinated, focused, multi-sectoral and multinational effort'.

The World Health Organisation (WHO) categorises antimicrobials used in human health as *critically important*, *highly important* and *important* to human health. Critically important antimicrobials (CIAs) should NOT be used as first line of treatment in animals –they should only be used when there is no effective alternative antimicrobials available for target species and indication–.

Additionally, some CIAs are further classified as Highest Priority Critically Important Antibiotics (HP-CIAs). WHO Highest Priority CIA group contains antimicrobials licensed for use in veterinary medicine including 3rd and 4th generation cephalosporins, fluoroquinolones, macrolides, and polymyxins. Further information is available in the DAFM webpage (Department of Agriculture, Food and the Marine, 2018).

Every year, the Veterinary Laboratory Service (VLS) handles between 1500–2000 bacterial isolates from multiple species; in 2018, there were 1,826 isolates. As part of DAFM plan to monitor AMR within the Irish animal health sector, VLS focuses on particular patterns of AMR, outlined in Figure 2.

A number of antibiotic panels are used across VLS to initially assess levels of AMR nationally, the Respiratory, Enteric, Gram-positive and the Mastitis causing Gram-negative panels (Figure 3). Statuary surveillance of AMR in zoonotic and commensal bacteria is carried out by CVRL as part of an EU- wide harmonised monitoring programme , details of which can be found here.

Figure 1: Antibiotic sensitivity test illustrating thin paper disks containing antibiotic placed on an agar plate with growing bacteria. Note the clear *zone of inhibition*, where bacteria growth around antibiotic disk has been inhibited. Photo: Pat Sheehan.
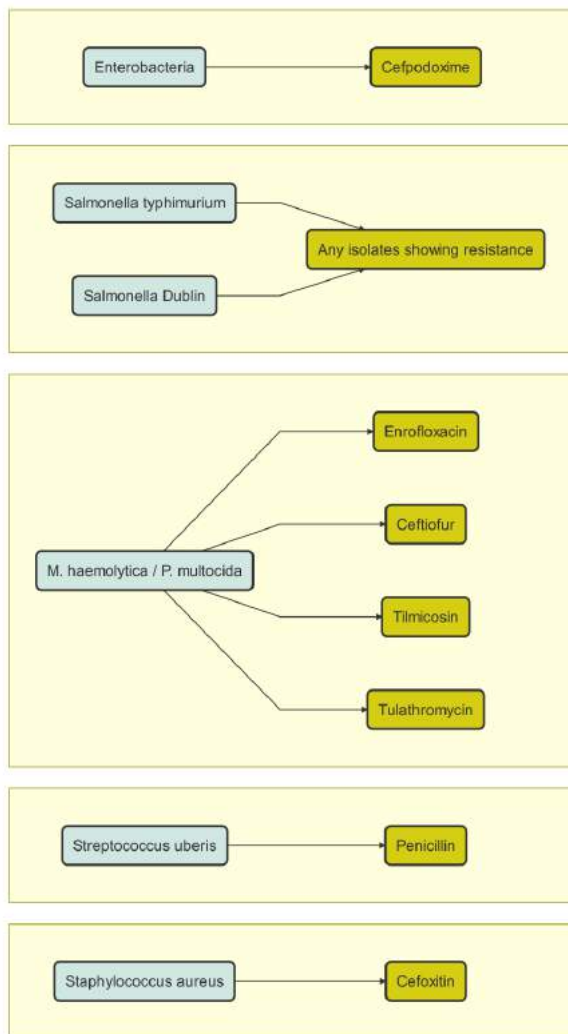
Disk Diffusion Test

The Kirby-Bauer test or disk diffusion test, is a standard tool for measuring the effectiveness of antimicrobials against pathogenic microorganisms (Figure 1). Antimicrobial-impregnated paper disks are placed on on a plate that is inoculated to allow growth of the bacteria and time for the agent to diffuse into the agar. As the drug moves through the agar, it establishes a concentration gradient. If the organism is susceptible to it, a clear zone will appear around the disk where growth has been inhibited.

The size of this *zone of inhibition* depends pon the sensitivity of the bacteria to the specific antimicrobial agent and the point at which the chemical's *minimum inhibitory concentration* (MIC) is reached.

Staphylococcus aureus

In 2018, there were 407 isolates of *Staphylococcus aureus* in the VLS. Of these, 392 (96.3 *per cent*) were susceptible to Tetracycline, 407 (100 *per cent*) were susceptible to Trimethroprim-Sulphonamide, 403 (99 *per cent*) were susceptible to Amoxycillin-Clavulanate

Figure 2: The significant AMR patterns that Veterinary Laboratory Service (VLS) monitors.

(Table 1).

Streptococcus uberis

In 2018, there were 291 *Streptococcus uberis* isolates in the VLS. Of these, 228 (78.3 *per cent*) were susceptible to Tetracyclines, 273 (93.8 *per cent*) were susceptible to Trimethoprim-Sulphonamide, 286 (98.2 *per cent*) was susceptible to Penicillin, 280 (96.2 *per cent*) susceptible to Ampicillin and 289 (99.3 *per cent*) were susceptible to Amoxycillin-Clavulanate (Table 1).

Table 1: Common bacterial isolates detected in the VLS and the level of sensitivity to first-line antibiotics

	S. aureus (n=407)	S. uberis (n=291)
Ampicillin	190 (46.6%)	280 (96.2%)
Tetracycline	392 (96.3%)	228 (78.3%)
Trimethoprim-Sulphonamide	407 (100%)	273 (93.8%)
Florfenicol	Not Tested	Not tested
Amoxycillin-Clavulanate	403 (99%)	289 (99.3%)

Case Study

Vancomycin resistant *Enterococcus spp.* (VRE) in a calf:

A single isolate of Vancomycin resistant *Enterococcus faecium* was confirmed in a calf using WGS. *Enterococci* are commensal Gram-positive bacteria that form part of the normal gut flora of animals and humans. Vancomycin is a member of the glycopeptide antimicrobial class and is of critical importance in the treatment of infections, caused by drug resistant organisms, such as *Staphylococcus aureus*. The use of the glycopeptide avoparicin, as a growth promoter in animals, was linked to the emergence of VRE in pigs and poultry and as a result, its use for this purpose was banned in 1996.

The level of VRE infection in Irish hospitals is one of the highest in Europe. Hospital outbreaks tend to be associated with a distinct, adapted subpopulation, which are often Ampicillin and Ciprofloxacin resistant. The latter is an unusual finding in animal derived isolates and the role of animals as a potential reservoir of resistance for human epidemic *Enterococcus* strains remains unclear.

Pasteurella multocida

In 2018, there were 181 isolates of *Pasteurella multocida* in the VLS. Of these, 173 (99.5 *per cent*) were susceptible to Tetracycline, 177 (97.7 *per cent*) was susceptible to Trimethoprim-Sulphonamide, 159 (87.8 *per cent*) were susceptible to Florfenicol, 179 (98.9 *per cent*) were susceptible to Amoxycillin-Clavulanate (Table 2).

Table 2: Common bacterial isolates detected in the VLS and the level of sensitivity to first-line antibiotics *(continued)*

	M. haemolytica (n=150)	P. multocida (n=181)
Ampicillin	143 (95.3%)	169 (93.4%)
Tetracycline	135 (90%)	173 (95.6%)
Trimethoprim-Sulphonamide	146 (97.3%)	177 (97.8%)
Florfenicol	149 (99.3%)	159 (87.8%)
Amoxicillin-Clavulanate	149 (99.3%)	179 (98.9%)

Case Study

AMR in *Pasteurella multocida*:

Pasteurella multocida frequently exists as a commensal bacterium of the respiratory tract in animals but it may act also as a primary or secondary pathogen. In 2018, almost 90 *er cent* of *P. multocida* isolates were susceptible to all of the antimicrobials in the respiratory panel (Ampicillin, Amoxicillin-Clavulanate, Ceftiofur, Tetracycline, Sulfamethoxazole-Trimethoprim, Enrofloxacin, Florfenicol, Tulathromycin and Tilmicosin).

To date, 1 multi-drug resistant isolate of *P. multocida* from 2018 has undergone WGS. Resistance genes for Aminglycoside, Macrolide, Sulphonamide, and Tetracycline resistance were identified in the strain, which originated from the lung tissue of a weanling calf. The calf was one of at least seven that died during a large outbreak of respiratory disease and it had concurrent lungworm infestation. It is planned to continue with the genotypic characterisation of resistant *P. multocida* in 2019.

Case Study

Multiple Drug Resistant *Escherichia coli* on a suckler farm:

Escherichia coli was isolated from the organs and faeces of animals from a farm that was experiencing high mortality rates due to cases of enteritis and unresponsive post- operative infections. Evaluation of the bacterial genome confirmed that the strain was pathogenic to cattle and that its virulence genes were not targeted by commercially available vaccines.

Genes encoding resistance to beta lactam antimicrobials, included extended spectrum Beta-lactams, Fluoroquinolones, Phenicols, Aminoglycosides, Sulphonamides and Tetracyclines were identified.

Table 3: Common bacterial isolates detected in the VLS and the level of sensitivity to first-line antibiotics *(continued)*

	E. coli (n=268)
Ampicillin	Not tested
Tetracycline	191 (71.2%)
Trimethoprim-Sulphonamide	230 (85.8%)
Florfenicol	Not Tested
Amoxycillin-Clavulanate	229 (85.4%)

Mannheimia haemolytica

In 2018, there were 150 isolates of *Mannheimia haemolytica*. Of these, 135 (90 *per cent*) was susceptible to Tetracycline, 146 (97.3 *per cent*) were susceptible to Trimethoprim-Sulphonamide and 149 (99.3 *per cent*) were susceptible to Amoxycillin-Clavulanate and Florfenicol (Table 2).

Escherichia coli

In 2018, there were 268 isolates of *Escherichia coli* in the VLS. Of these, 191 (71.2%) were susceptible to Tetracycline, 230 (85.8%) were susceptible to Trimethoprim-Sulphonamide and 229 (85.4%) were susceptible to Amoxycillin-Clavulanate (Table 3).

Figure 3: Antibiotic panels used in the VLS and their constituent antibiotics

Respiratory Panel

- Ampicillin
- Amoxicillin Clavulanate
- Ceftiofur
- Tetracycline
- Sulfamethoxazole & Trimethoprim
- Enrofloxacin
- Florfenicol
- Tulathromycin
- Timlicosin

Enteric Panel

- Ampicillin
- Amoxicillin Clavulanate
- Ceftiofur
- Tetracycline
- Sulfamethoxazole & Trimethoprim
- Enrofloxacin
- Neomycin
- Cefpodoxime
- Streptomycin

Gram-Positive

- Ampicillin
- Amoxicillin Clavulanate
- Ceftiofur
- Tetracycline
- Sulfamethoxazole & Trimethoprim
- Cephalothin
- Cefoxitin
- Pirlimycin
- Kanamycin
- Erythromycin
- Cephalexin & Kanamycin

Mastitis Causing Gram-Negative Panel

- Ampicillin
- Amoxicillin Clavulanate
- Ceftiofur
- Tetracycline
- Sulfamethoxazole & Trimethoprim
- Enrofloxacin
- Neomycin
- Streptomycin
- Kanamycin
- Cefpodoxime
- Cephalexin & Kanamycin

Maldi-ToF

One of the more recent advancements in clinical veterinary bacteriology which has helped immensely in relation to identification of pathogens is the use of Maldi-ToF Mass Spectrometry. MALDI is the abbreviation for Matrix Assisted Laser Desorption/Ionization, TOF is Time of Flight. A portion of a colony of the microbe in question is placed onto the sample target and overlaid with matrix. The mass spectra generated are analyzed by dedicated software and compared with stored profiles. Species identification by this procedure is much faster, more accurate and cheaper than other procedures based on immunological or biochemical tests. Maldi ToF can also be used to predict the antibiotic susceptibility of a bacterial species. In 2018, this technique was used to confirm the identity of 588 isolates within DAFMs Veterinary Laboratory Service.

Figure 3: Sequencing platform for the generation of whole genome sequences. Photo: Rosemarie Slowey.

Whole Genome Sequencing

Sequencing of bacterial DNA sequences (whole genome sequencing, WGS) has been undertaken in the VLS since 2016. These sequences can be screened for antimicrobial resistance and virulence genes and sequences compared to evaluate the relatedness of the strains, which is particularly important in outbreak investigations. WGS has been used to confirm unusual resistance patterns initially detected in the RVLs.

References

Department of Agriculture, Food and the Marine (2018). "Policy on Highest Priority Critically Important Antimicrobials." URL https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/amr/PolicyHighestPriorityCriticallyImpAntimicrobials191118.pdf.

Bovine Parasitic Disease

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Parasitic disease is consistently one of the most frequent *post-mortem* diagnosis in Regional Veterinary Laboratories. In addition, faecal samples are submitted for clinical diagnosis; the majority of these samples come from herds or flocks with animals showing clinical signs of diarrhoea and/or weight loss. *Fasciola hepatica* (Fasciolosis),Trichostrongylidae (Parasitic Gastro-Enteritis) and *Dictocaulus viviparus* (Parasitic Pneumonia) are the most important organisms responsible for parasitic disease in cattle and sheep.

Faecal samples submitted to DAFM laboratories are routinely examined for eggs (Trichostrongylidae [1], *Fasciola hepatica* and *C. daubneyi*), lungworm larvae (*Dictyocaulus viviparus*), and coccidial oocysts (Figure 1).

Trichostrongylidae

Ostertagia ostertagi and *Cooperia oncophora* are the most prevalent nematodes affecting cattle in the Republic of Ireland (Murphy *et al.*, 2006). These parasites can cause parasitic gastro-enteritis with acute clinical disease significantly reducing productivity of affected animals. *O. ostertagi* triggers two different syndromes in weanlings: Ostertagiosis *Type 1* which typically occurs in late summer or autumn after a mass emergence of larvae on fields.
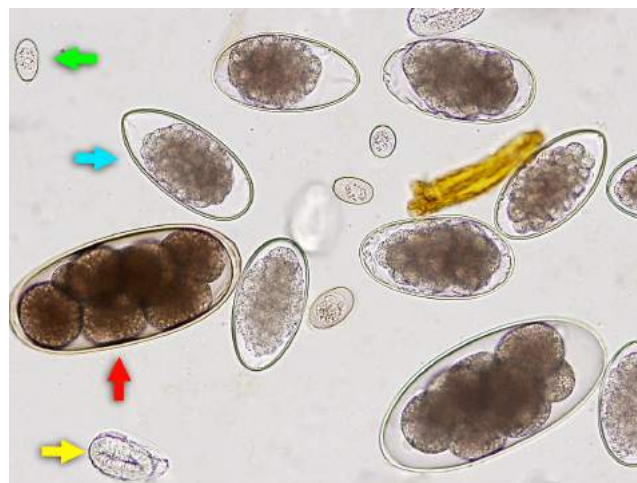
Table 1: Charateristic comparison of the two most prevalent nematodes affecting cattle in the Republic of Ireland.

Cooperia oncophora	*Ostertagia ostertagi*
Small intestine	Glands of abomasum
Adult stage 15-18 days PI*	Adult stage 18-21 days PI
Immunity develops after first grazing season	Immunity develops after second grazing season

[*] *Post-Infection*

Ostertagiosis *Type 2*, observed especially in late winter and early spring and caused by larvae which experienced a delayed development in abomasal mucosa (hypobiosis). Ostertagiosis *Type 2* usually shows poor response to treatment; however, it can be prevented by appropriate treatment at time of housing. In general, cattle develop immunity to parasites (Table 1);

[1]The *Trychostrongylidae* superfamily includes the Nematodes *Ostertagia*, *Teladorsagia*, *Cooperia*, *Trichostrongylus*,*Haemonchus* and *Nematodirus*.

Figure 1: Modified McMaster fecal egg counting. Microscopic appearance of parasitic eggs and oocysts in a faecal smear: *Trichostrongyles* (blue arrow), *Nematodirus* (red arrow), Larvated *strongyloid* egg (yellow arrow) and *coccidial oocysts* (green arrow). Photo: Cosme Sánchez-Miguel.
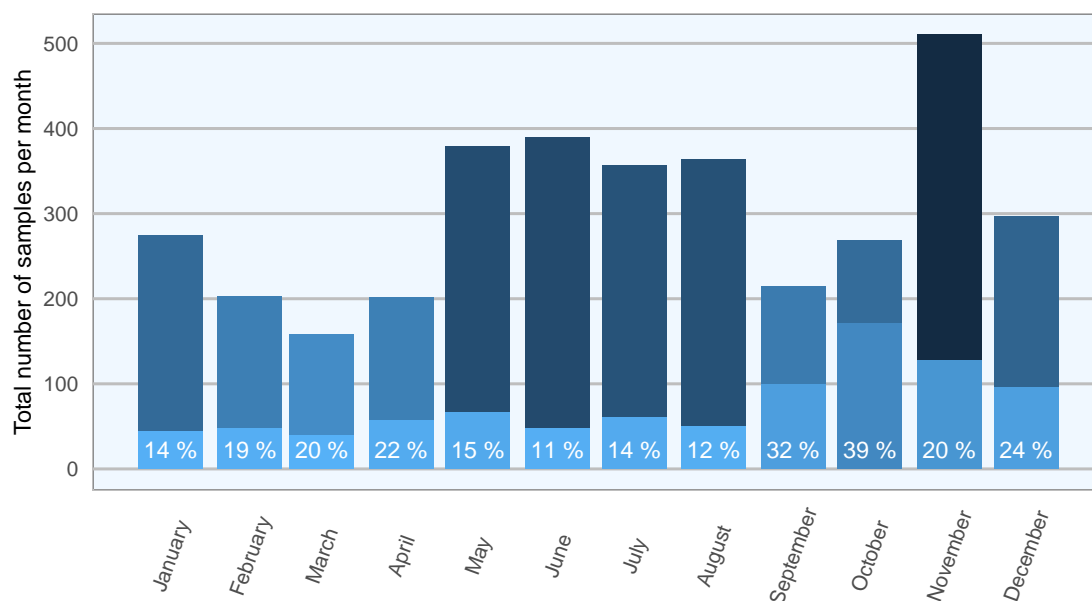
this immunity does not prevent infection but halts development of clinical disease.

Table 2: Number of bovine faecal samples tested for Trichostrongylidae eggs in 2018 and results by percentage (n= 4530).

Result	No. of samples	Percentage
Negative	3625	**80.0**
Low (50-500 epg)	683	**15.1**
Medium (500-1200 epg)	132	**2.9**
High (>1200 epg)	90	**2.0**

In the last decade, it has been shown that cows can develop a subclinical parasitic infection with *O. ostertagi* which can lead to significant production losses linked to reduced milk yields, weight loss, non-specific immune suppression and mortality (Delafosse, 2013).

Faecal samples for investigation of parasitic burden in cattle were received throughout the year with peaks from May to August and in November (Figures 2 and 3). The peak during summer months covers the traditional grazing season and is most likely due to monitoring efforts. The high number of submissions in Novem-

Figure 2: Stacked count of bovine faecal samples (all ages) tested per month for Trichostrongylidae during 2018. The percentage in each bar represents positive samples (n= 4530).

ber coincides with the beginning of housing, which commonly associates with antiparasitical treatment. The highest amount of samples yielding positive results occurred in spring, late autumn and early winter (Figures 2 and 3).
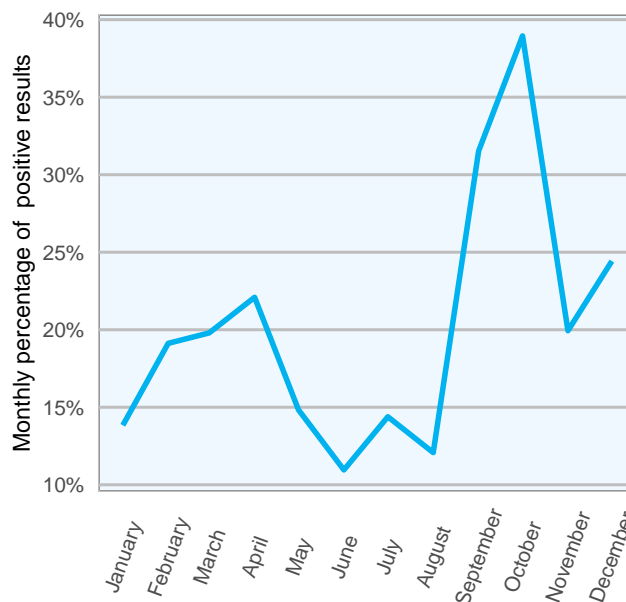
Nematodirus spp.. Also called *thread necked worms* are a species of nematodes which mainly affect small ruminants, but have been reported to cause disease in cattle.

Table 3: Number of bovine faecal samples for *Nematodirus* eggs in 2018 and results by percentage (n= (n= 4529).

Result	No. of samples	Percentage
Negative	4441	**98.1**
Low (50-500 epg)	77	**1.7**
Medium (500-1200 epg)	8	**0.2**
High (>1200 epg)	3	**0.1**

Nematodirus battus is the most significant species in Ireland and affects mainly naive young lambs. *N. helvetianus*, which is more likely to infect cattle, has been noted across Europe but appears to be more common across Australia and Asia (McMahon *et al.*, 2017).

In 2018, there was an an insignificant number of bovine faecal samples were *Nematodirus* eggs were present (Table 3).

Figure 3: Percentage of positive bovine faecal samples for Trichostrongylidae eggs in 2018 (n= 4530).

Coccidia

Coccidia (*Eimeria spp.*) are protozoa (Figure 4) which can cause significant clinical disease in calves and lambs from 3 *weeks* to 9 *months* of age. Diarrhoea and dysentery are common signs of clinical coccidiosis. While there is a wide range of coccida species, only three species are considered pathogenic in cattle: *Eimeria bovis*, *Eimeria zuerni* and *Eimeria alabamensis*. Coccidia are host specific.

Table 4: Comparison of the pre-patent periods of *Eimeria spp.*

Eimeria	Pre-patent period*
Eimeria bovis	21 days
Eimeria zuerni	21 days
Eimeria alabamensis	8-12 days

Note:
* Time between infection of the animal and the first appearance of oocysts in faeces

- Coccidia have a life cycle of 21 *days*. Examining calf faeces for oocysts before 21 *days* of age will produce a false negative result.
- Oocyst production in clinical cases of coccidiosis may be low. Therefore, it is a good idea to sample comrades which are as yet unaffected.

Clinical disease in adults is rather uncommon as immunity to the disease can develop quickly. Disease is usually self-limiting lasting 3–4 *weeks*, the time frame for parasites to complete their life cycle in host. Infection occurs after ingestion of sporulated oocysts, these release sporozoites that penetrate the epithelial cells of small and large intestine. Further development continues within epithelial cells where coccidia mature and produce oocysts for excretion in faeces; affected epithelial cells are destroyed leading to rapid and severe damage of intestinal epithelium.

As in previous years, in 2018 the majority of positive samples contained low numbers of oocysts and would not be considered of clinical significance (Table 5).

Table 5: Number of bovine faecal samples submitted in 2018 (all ages) for detection of coccidial oocysts and results by percentage, (n= 4506).

Result	No. of samples	Percentage
Not Detected	3536	78
Light Infection	712	16
Moderate Infection	148	3
Heavy Infection	71	2
Severe Infection	39	1

Figure 4: Microscopic appearance of coccidial oocyst in a faecal smear (light microscope at 100x-Oil). Photo: Cosme Sánchez-Miguel.
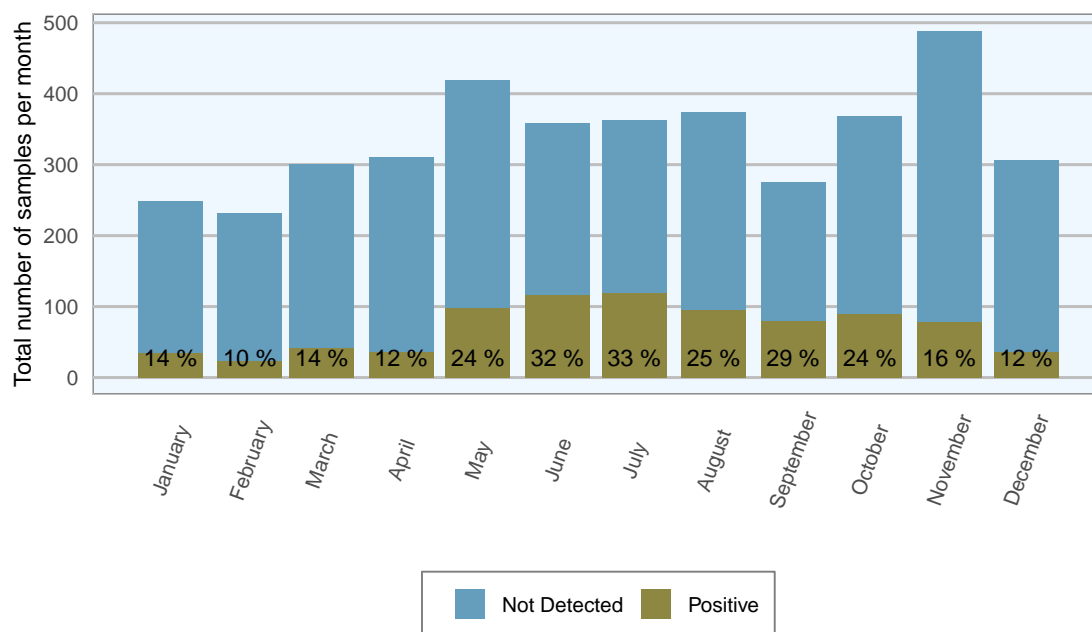
Avoidance of faecal contamination of feed and water, maintenance of dry bedding, not mixing age groups and, if necessary, the use of prophylacric anticoccidian drugs help to protect calves from exposure to large amounts of coccidial oocysts. This enables calves to acquired immunity without development of clinical signs and loss in production.

Treatment and control. Anti-protozoal treatment at the occurrence of clinical signs is usually unrewarding as diarrrhoea indicates the end of coccidia life cycle in host and that severe intestinal damage is already present.
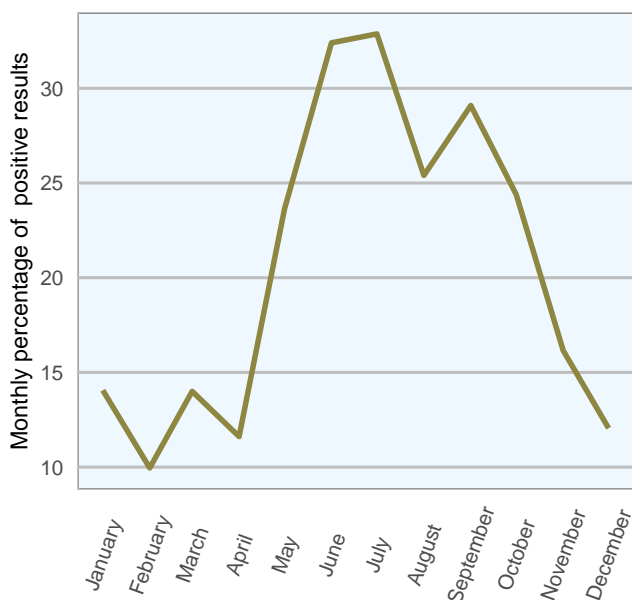
Consequently, where high environmental contamination is present, control is best achieved by hygiene and prophylactic treatment.

Currently, there a three anticoccidials registered for the use in bovine in the Republic of Ireland:

- Decoquinate: 60.6 *g/kg* premix for medicated

Figure 5: Stacked number of bovine faecal samples (all ages) tested for coccidial oocysts in 2018. The percentage in each bar represents the number of positives (n= 4506).

Figure 6: Count of bovine faecal samples exmined for coccidial oocysts in 2018 (n= 4506).

feeding stuff
- Diclazuril: 2.5 *mg/ml* oral suspension
- Toltrazuril: 50 *mg/ml* oral suspension

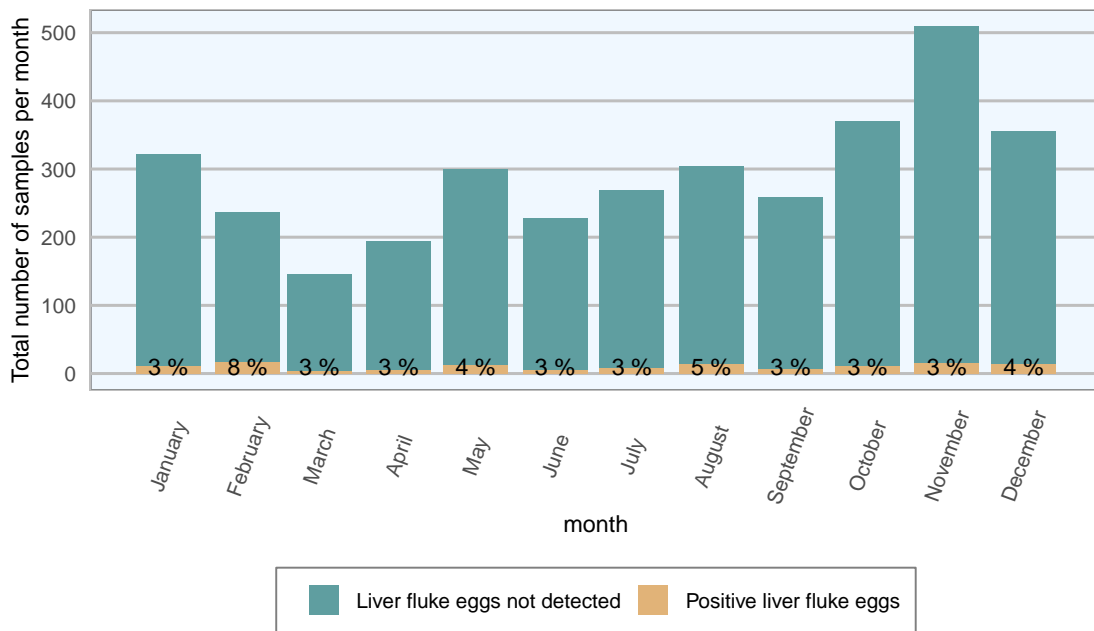For further information please refer to the manufacturers' advice.

Rumen and Liver Fluke

The most relevant trematodes in Ireland are liver fluke species *Fasciola hepatica* and rumen fluke species *Calicophoron daubneyi*. If measured by the detection of eggs in faecal samples, in recent years the prevalence of *Fasciola hepatica* appears to have declined, whereas rumen fluke appears to stay static (All-island Disease Surveillance Report, Department of Agriculture, Food and the Marine 2018, pp. 46–47).

Table 6: Number of bovine faecal samples submitted in 2018 (all ages) for detection of liver fluke eggs and results by the percentage (n= 3853).

Result	No. of samples	Percentage
Liver fluke eggs not detected	3722	**97**
Positive liver fluke eggs	131	**3**

Fasciola hepatica causes disease in cattle mainly during the chronic phase of the infestation, when adults

Figure 7: Stacked number of bovine faecal samples (all ages) tested for liver fluke in 2018. The percentage in each bar represents the number of positive samples per month (n= 3853).

trematodes inhabit liver tissue. Clinical signs include weight loss, diarrhoea and hypoproteinaemia due to liver damage and epithelial loss in the intestine.
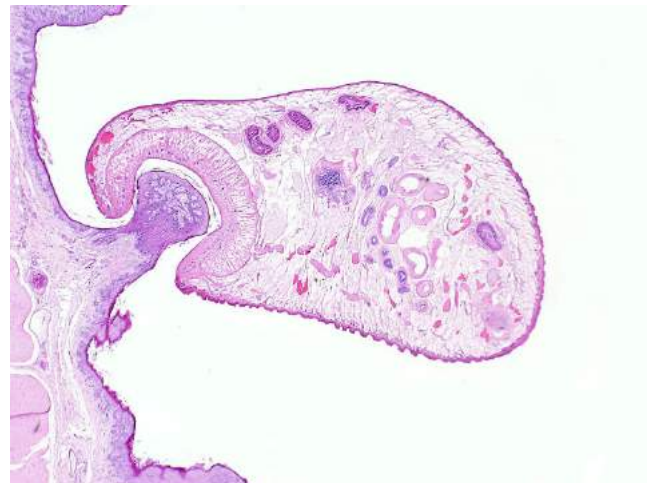
Risk of infection with both parasites varies from year to year depending on climatic conditions, especially rainfall and surface moisture. Wet ground conditions at moderate temperatures, as occurring in Ireland during spring and summer, particularly favour reproduction and spread of the mollusk intermediary host (*Galba trunculata*), development of fluke in intermediary host and shedding of the metacercaria on pasture.

Every autumn, to assist farmers and private veterinary practitioners, DAFM issues a fluke forecast based on analysis of meteorological data for the preceding 6 *months*.

In 2018, as in previous years, rumen fluke eggs were more often detected in bovine faecal samples than liver fluke eggs.
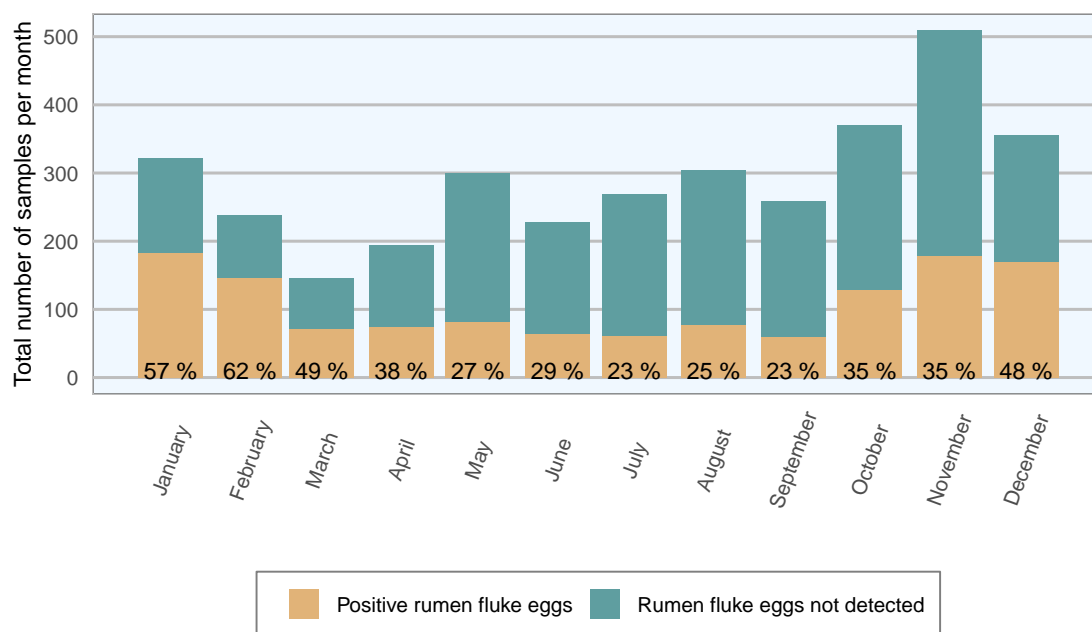
Table 7: Presence of Rumen fluke eggs in bovine faecal samples in 2018 (n= 3853).

Result	No. of samples	Percentage
Rumen fluke eggs not detected	2453	64
Positive rumen fluke eggs	1400	36

Rumen fluke is mainly described to cause disease

Figure 8: Microphotograph of a Rumen fluke (*Calicophoron daubneyi*) attached to the rumen wall by its oral sucker. Photo:Cosme Sánchez-Miguel.

Figure 9: Stacked count of bovine faecal samples (all ages) tested for rumen fluke. The percentage in each bar represents positive samples (n= 3853).

during the juvenile phase of the parasite, when it inhabits the duodenum from several weeks to months, attaching to duodenal wall. Severe disease outbreaks have been described when young animals were exposed to large number of metacercariae in the field (O'Shaughnessy *et al.*, 2018). However, cases of clinical disease are very rare with only 3– 4 cases being reported annually in Ireland (Toolan *et al.*, 2015).

To date, adult rumen fluke living in the rumen and attaching to ruminal mucosa are not thought to cause significant disease, however there is some evidence of inflammatory changes occurring in reticulum and rumen during infestation.

Both, liver and rumen fluke parasites, use the same molluscan intermediary host, the mud snail (*Galba trunculata*), possibly leading to an interspecific competition between larval stages of the two species, which might have an additional impact on the relative abundance of infection. Further research might be needed to elucidate this further (Deplazes *et al.*, 2016).

Treatment and Control. There is a variety of flukicides available for treatment and control of *Fasciola hepatica*. However, due to regulatory changes in recent years, restrictions apply for use in dairy cattle depending on the active ingredient and the reproductive stage of the animals. Further on, when choosing a suitable treatment, consideration of the developmental stage of the parasite is needed as only some products affect juvenile stages (e.g. Triclabendazole, Closantel, Nitroxinyl) whereas other products only affect the adult stage (>12 *weeks*) of the parasite. Depending on the product and the time of application (e.g. at housing) a repeat or delay in treatment might be appropriate.

- Sedimentation method detects both eggs, *Fasciola hepatica* (liver fluke) and *Calicophoron daubneyi* (rumen fluke).

- The significance of *rumen fluke* eggs in cattle faeces is difficult to determine, adults are not considered to be pathogenic while larvae are. If eggs are found in a number of animals with poor thriving, treatment may be warranted. Alternatively, if eggs are found in healthy cattle in good condition, it may be wise just to monitor thrive.

Finally, there is also evidence that there is emerging resistance against some flukicides , in particular Triclabendazole, consequently, the choice of appropriate flukicide, needs to be prudent and circumspect (Kelley *et al.*, 2016). A comprehensive list of available

flukicides, including restrictions, is available from the Irish Health Products Regulatory Authority (HPRA). To date, there is no registered treatment for rumen fluke (*C. daubneyi*) available. However, there has been evidence that some products like oxyclozanide and closantel show good activity against adult rumen fluke infestation and lead to a reduction in faecal egg count. Dose rates and related side effects, as well as route of administration, appear to have an impact on treatment success (Arias *et al.*, 2013; Malrait *et al.*, 2015).

References

Arias M, Sanchís J, Francisco I, Francisco R, Piñeiro P, Cazapal-Monteiro C, Cortinas F, Suarez J, Sanchez-Andrade R, Paz-Silva A (2013). "The efficacy of four anthelmintics against Calicophoron daubneyi in naturally infected dairy cattle." *Veterinary parasitology*, **197**(1-2), 126–129. URL http://www.sciencedirect.com/science/article/pii/S0304401713003622.

Delafosse A (2013). "The association between Ostertagia ostertagi antibodies in bulk tank milk samples and parameters linked to cattle reproduction and mortality." *Veterinary Parasitology*, **197**(1), 212 – 220. ISSN 0304-4017. doi:https://doi.org/10.1016/j.vetpar.2013.05.023. URL http://www.sciencedirect.com/science/article/pii/S0304401713003464.

Department of Agriculture, Food and the Marine (2018). "Policy on Highest Priority Critically Important Antimicrobials." URL https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/amr/PolicyHighestPriorityCriticallyImpAntimicrobials191118.pdf.

Deplazes P, Eckert J, Mathis A, von Samson-Himmelstjern G, Zahner H (2016). *Parasitology in veterinary medicine.* Wageningen Academic Publishers.

Kelley JM, Elliott TP, Beddoe T, Anderson G, Skuce P, Spithill TW (2016). "Current threat of triclabendazole resistance in Fasciola hepatica." *Trends in parasitology*, **32**(6), 458–469.

Malrait K, Verschave S, Skuce P, Loo HV, Vercruysse J, Charlier J (2015). "Novel insights into the pathogenic importance, diagnosis and treatment of the rumen fluke (Calicophoron daubneyi) in cattle." *Veterinary Parasitology*, **207**(1), 134 – 139. ISSN 0304-4017. doi: https://doi.org/10.1016/j.vetpar.2014.10.033. URL http://www.sciencedirect.com/science/article/pii/S0304401714005603.

McMahon C, Edgar HWJ, Barley JP, Hanna REB, Brennan GP, Fairweather I (2017). "Control of Nematodirus spp. infection by sheep flock owners in Northern Ireland." *Irish Veterinary Journal*, **70**(1), 31. ISSN 2046-0481. doi:10.1186/s13620-017-0109-6. URL https://doi.org/10.1186/s13620-017-0109-6.

Murphy T, Fahy K, McAuliffe A, Forbes A, Clegg T, O'Brien D (2006). "A study of helminth parasites in culled cows from Ireland." *Preventive Veterinary Medicine*, **76**(1), 1 – 10. ISSN 0167-5877. doi: https://doi.org/10.1016/j.prevetmed.2006.04.005. URL http://www.sciencedirect.com/science/article/pii/S016758770600078X.

O'Shaughnessy J, Garcia-Campos A, McAloon CG, Fagan S, de Waal T, McElroy M, Casey M, Good B, Mulcahy G, Fagan J, et al (2018). "Epidemiological investigation of a severe rumen fluke outbreak on an Irish dairy farm." *Parasitology*, **145**(7), 948–952. doi:10.1017/S0031182017002086.

Toolan DP, Mitchell G, Searle K, Sheehan M, Skuce PJ, Zadoks RN (2015). "Bovine and ovine rumen fluke in Ireland—Prevalence, risk factors and species identity based on passive veterinary surveillance and abattoir findings." *Veterinary Parasitology*, **212**(3), 168 – 174. ISSN 0304-4017. doi: https://doi.org/10.1016/j.vetpar.2015.07.040. URL http://www.sciencedirect.com/science/article/pii/S0304401715003829.

Bovine Viral Diarrhoea (BVD) Eradication Programme and Infectious Bovine Rhinotracheitis

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[a]BVD & IBR Programme Manager, Animal Health Ireland, 4–5 The Archways, Carrick on Shannon, Co. Leitrim

Bovine Viral Diarrhoea (BVD) is a highly contagious disease in cattle that can spread directly from infected animals or indirectly (e.g. via slurry, equipment, farm workers/visitors). Most BVD infections are Transient Infections (TI) without clinical signs. BVD affects fertility, calf health and causes foetal losses (see below for further details). Infection with BVD virus within the first 120 days of pregnancy may result in a persistently infected foetus. Persistently Infected (PI) animals will shed high levels of BVD virus for life; they are, therefore, the most significant source of virus.

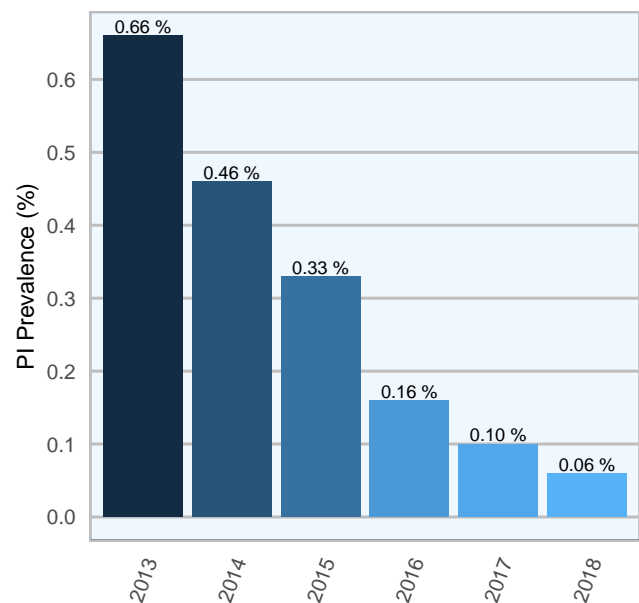## Bovine viral diarrhoea (BVD) eradication programme

Just over 2.3 million calves were born in 2018. Consistent with previous years, there was a very high level of compliance with requirement to tissue tag test newborn calves, with results available for 99.5 *per cent* of these calves. Prevalence of *PI* births in 2018 continued to decline, only 0.06 *per cent* of calves tested (1,324) were found to be persistently infected (*PI*) with BVD virus (Table 1 and Figures 1 and 4). This represents a reduction of approximately 44 *per cent* compared to 2017, when 0.10 *per cent* (2,335) were identified as *PI*, and of 90 *per cent* when compared to 2013, first year of the compulsory national programme, when 0.66 *per cent* (13,877) were PIs (Figure 3). Prevalence of herds where one or more calves had a positive or inconclusive result also decreased by 50 *per cent*, from 2.02 *per cent* (1,577) in 2017 to 1.1 *per cent* (805) in 2018 (Table 2 and Figure 2).

Table 1: Animal- level prevalence of PI calves born during each year of the programme by herd type.

Year	Total	Beef	Dairy	Dual
2013	0.66	0.78	0.55	0.82
2014	0.46	0.54	0.37	0.58
2015	0.33	0.39	0.26	0.54
2016	0.16	0.22	0.12	0.27
2017	0.10	0.13	0.08	0.20
2018	0.06	0.07	0.04	0.11

Programme enhancements introduced by the BVD Implementation Group (BVDIG), in combination with a decreasing prevalence, have had a significant impact on the number of *PIs* alive relative to the same time point in previous years. These enhancements, funded through the Rural Development Plan under the Targeted Advisory Service on Animal Health (TASAH), include increased levels of financial support for removal of *PIs* within a reduced period of time, automation of imposition of restrictions in herds retaining *PI* calves for more than five weeks after the date of their first test and mandatory herd investigations within three months of result disclosure.

Figure 1: Animal- level prevalence of PI calves born during each year of the programme.

Collectively, these measures have helped to achieve the lowest numbers of *PIs* alive recorded in the programme to date. At the end of 2018, only 33 *PIs* born during the year were still alive, compared to a figure of 53 at the end of 2017.

Programme enhancements have also contributed to reducing the number of herds retaining *PIs*. Only 8 herds were retaining *PIs* at the end of 2018 (no registered date of death within 5 weeks of the date of initial

test), compared to 16 herds at the same point in 2017. While this clearly demonstrates good progress, it is critical that incidence of retention is reduced to zero, with all calves being tested as soon as possible after birth and *PIs* removed as rapidly as possible thereafter.

Negative herd status (NHS)

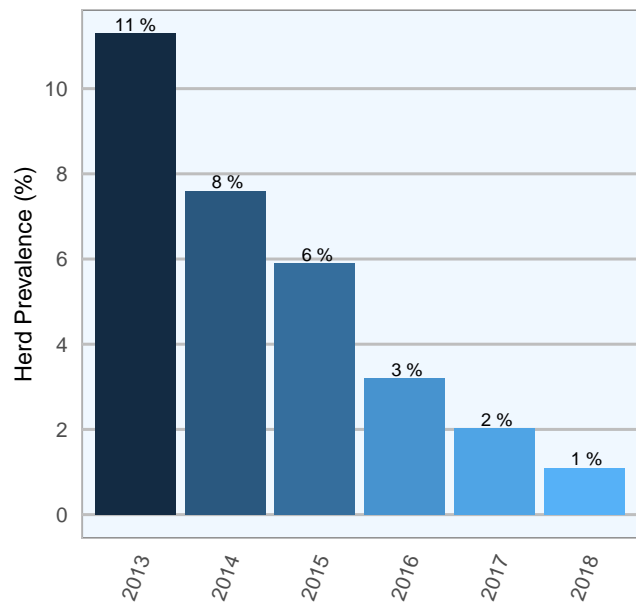A herd may qualify for negative herd status (NHS) by meeting the following requirements:

1. Existence of a negative BVD status for every animal currently in the herd, on the basis of either 'direct' or 'indirect' results.

2. Absence in the herd of animals deemed to be persistently infected with BVD virus in the 12 months preceding the acquisition of NHS.

Negative herd status (NHS). By the end of 2017, approximately 71,000 (86 *per cent*) of some 83,000 breeding herds had achieved NHS. By the end of 2018, this had risen to close to 75,000 (90 *per cent*), with a further 7,000 only being ineligible due to presence of a small number of untested animals. While this is an important milestone for any herd, NHS also brings an economic benefit, as a number of laboratories that use the RTPCR test method offer testing at reduced costs to NHS herds.

Table 2: Herd- level prevalence of PI calves born during each year of the programme by herd type.

Year	Total	Beef	Dairy	Dual
2013	11.3	8.80	20.3	13.8
2014	7.6	6.00	13.1	10.7
2015	5.9	4.50	10.4	9.3
2016	3.2	2.40	5.7	5.6
2017	2.0	1.30	3.8	4.0
2018	1.1	0.72	2.1	2.2

The BVD status of almost all animals (99.5 *per cent*) in the 83,000 breeding herds in Ireland is now known; main exception is a decreasing number of animals, born before the start of the compulsory programme in 2013, that have neither been tested nor produced a calf. At the end of 2018 the number of these animals was approximately 6,000. The majority of these animals are in beef herds, are male or have not had a calf registered to them. These animals are not required to be
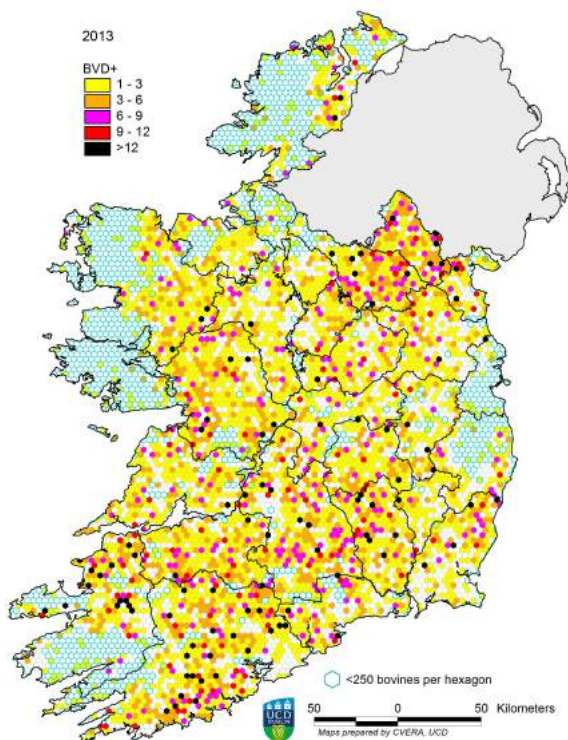

Figure 2: Herd- level prevalence of PI calves born during each year of the programme.

tested under current legislation and may presently be sold untested. It is important that these animals are tested as, on occasion, *PI* animals have been identified within cohort. During the summer of 2018 AHI contacted by SMS herds containing pre-2013-born animals to encourage their testing.

The number of animals born since January 2013 that do not have a valid test result and are, therefore, not compliant with legislation requirements has also been reduced to approximately 11,000. The majority have never been tested, whilst a small number has had an initial empty result and were not retested; most of these animals are born in 2018 (85 *per cent*), with remaining 15 *per cent* from preceding years. During 2018, DAFM issued letters to these herds notifying them of the need to test these animals.

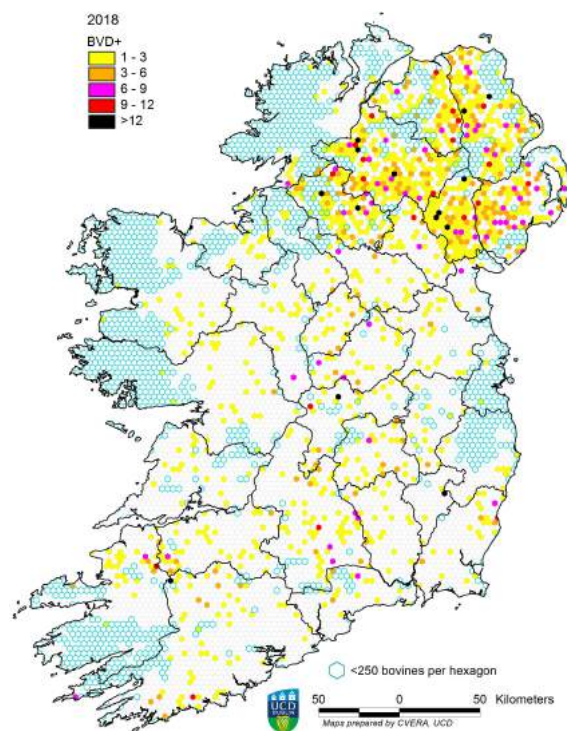**Targeted Advisory Service on Animal Health (TASAH).** Since 2017, all herds with positive results are required to undergo a herd investigation by a trained veterinary practitioner within 3 months of the initial positive result. These investigations, conducted through the Targeted Advisory Service on Animal Health (TASAH) and funded through the Rural Development Plan (2014–2020), seek to review herd biosecurity, identify a plausible source or sources of infection, ensure that herds are left free from BVDV and agree farm-specific mea-

Figure 3: Distribution of calves born persistently infected (PI) with BVD virus in 2013. Each hexagon covers an area of approximately 10 km^2. Hexagons in which there are fewer than 250 cattle (e.g. mountainous/urban areas and lakes) are shown with blue border.

Figure 4: All-island distribution of calves born persistently infected (PI) with BVD virus in 2018.

sures to prevent its re-introduction. By the end of 2018, 699 investigations had been completed, biosecurity recommendations provided to herd owners and results reported to **?**.

Preliminary analysis of these results indicated that the majority (91 *per cent*) of herd owners were provided with three biosecurity recommendations, most commonly related to risks of introduction of virus associated with personnel (including farmers), purchase of cattle, contact with neighbouring cattle at pasture and role of vaccination. One or more plausible sources of infection were identified in 74 *per cent* of herds, with a single plausible source identified in 43 *per cent* of herds. In 43 *per cent* of cases, the source was considered to be within the herd, while in 57 *per cent* of cases it was outside the herd. The most commonly identified plausible sources of infection were contact at boundaries, introduction of transiently infected animals without adequate quarantine, retained *PI* animals, personnel (including farmers), absence of appropriate hygiene measures and trojan dams. These data provide a basis
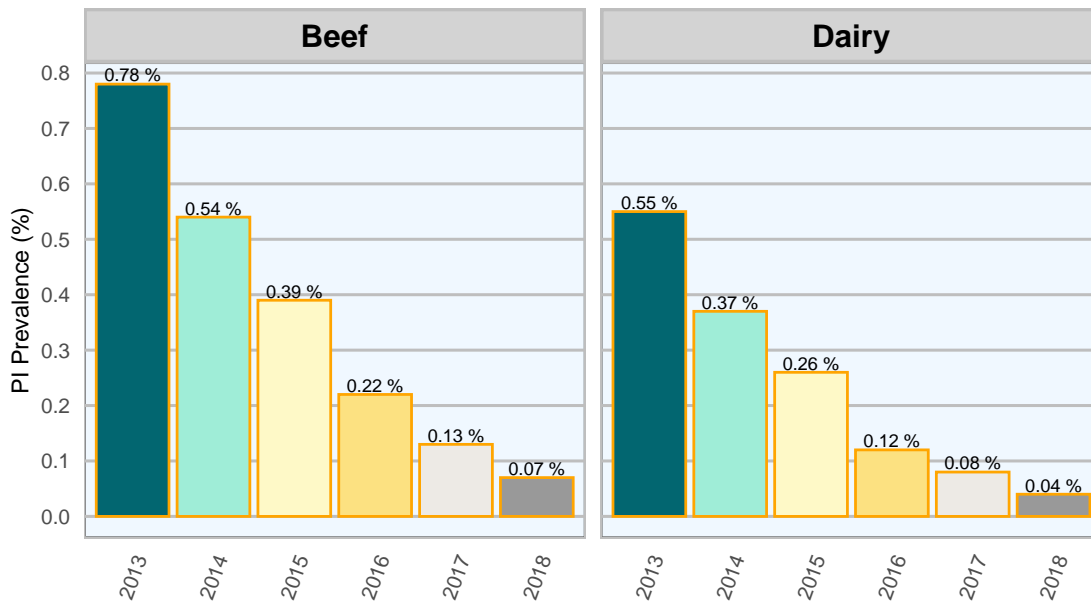
for targeted biosecurity advice to prevent accidental introduction of BVD virus to herds that are currently free of infection.

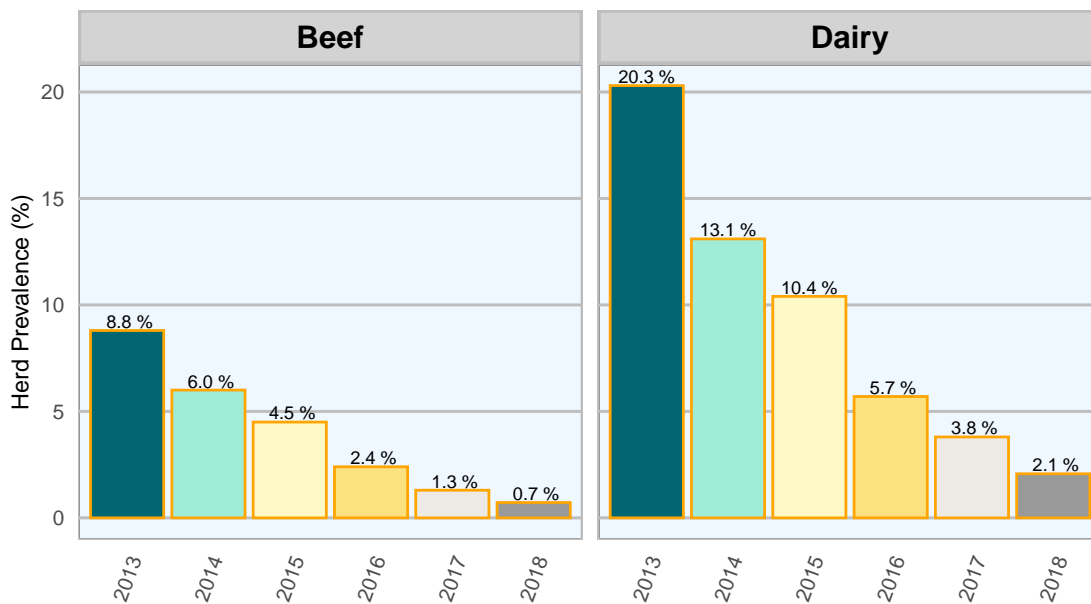## Infectious bovine rhinotracheitis eradication programme

Infectious Bovine Rhinotracheitis (IBR) is caused by a virus called Bovine Herpes Virus-1 (BoHV-1) that spreads between cattle and usually causes inflammation in nose and upper airways. Only primary infections are commonly associated with any clinical signs and severity can vary from inapparent to very severe. Latent infection refers to a carrier state where virus survives in an infected animal, though not causing disease or spreading). All animals that have ever had a primary infection are considered to be latently infected. Reactivation of latent infections provides a source of virus for new primary infections.

During 2018, the IBR Technical Working Group (TWG) developed and implemented the first phase of a pilot IBR Programme (**?**) for herds participating in Phase Three of the Teagasc/Irish Farmers journal BETTER Farm Beef Programme.

This pilot comprised application of an IBR on-farm veterinary risk assessment and management plan (VIBRAMP), sampling of herds and provision of biosecurity

Figure 5: Animal-level prevalence of BVDv PI calves born during each year of the programme by beef and dairy herds.

Figure 6: : Herd-level prevalence of BVDv PI calves born during each year of the programme by beef and dairy herds.

Figure 7: Fibrinonecrotic tracheitis caused by Infectious Bovine Rhinotracheitis (IBR). Photo: Cosme Sánchez-Miguel.

and disease control advice.

VIBRAMP consists of a questionnaire that captures details related to farm structure, animal movements, biosecurity and vaccination history, with vet and herd owner agreeing to up to three changes to improve biosecurity. A group of private veterinary practitioners was trained on this disease, application of the VIBRAMP and interpretation of test results. This was a mandatory requirement for vets to be able to participate in the programme.

In addition to VIBRAMP and formulation of biose-curity recommendations for those farms, the pilot programme included partial sampling of herds for IBR. Herds were tested by applying a 'snap shot' which required to sample 30 randomly selected over 9 *months*-old animals that are used, or intended to be used, for breeding. Samples were tested at DAFM Blood Testing Laboratory in Cork with an IBR *gE* (marker) ELISA. This sampling allowed a statistically-based estimation of the proportion of IBR-positive animals in a herd and formulation appropriate recommendations to farms in terms of IBR-control.

By the end of 2018, all of herds were sampled and tested and most had completed VIBRAMP. Results from this testing will be used to evaluate herd status, identify risk factors associated with presence of infection, identify common biosecurity risks and make informed decisions on further testing and vaccination. For example, testing of all animals in herds with a negative snap shot result would be justified, allowing identification and removal of any carrier animals and enabling herds to move rapidly to freedom from disease status. Information generated will also be used by the IBR TWG to inform options for a future IBR eradication programme for Ireland.

Diseases of Sheep

Veterinary Laboratory Service

2018

All-Island Animal Disease Surveillance Report

Sheep Diseases Overview

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All-Island Animal Disease Surveillance Report 2018 (compiled on October 3, 2019).

The Regional Veterinary Laboratories (RVLs) of the Department of Agriculture, Food and the Marine (DAFM) are engaged primarily in scanning (passive) surveillance by gathering data from *post-mortem* and clinical sample submissions. Analysis of this data provides an insight into trends of disease incidence and causes of mortality on Irish farms, thereby informing decision-making relevant to disease control at a national level. Tables and charts are generated with test results and *post-mortem* diagnoses from voluntary submissions of material (carcasses and clinical samples) to RVLs by farmers through their private veterinary practitioners (PVPs). Therefore, it should be noted that data reflects only those cases where the PVPs considered it appropriate to request a laboratory investigation and the herdowner was motivated to deliver the carcass to an RVL.

Table 1: Conditions most frequently diagnosed (top 15 categories) on *post-mortem* examinations of lambs in 2018 (n= 798).

Category	No. of Cases	Percentage
GIT Infections	170	21.3
Systemic Infections	97	12.2
Respiratory Infections	94	11.8
Clostridial disease	86	10.8
Diagnosis not reached	55	6.9
Nutritional/metabolic conditions	47	5.9
GIT torsion/obstruction	37	4.6
Navel Ill/Joint Ill	32	4.0
CNS	27	3.4
Urinary Tract conditions	18	2.3
Liver disease	17	2.1
Perinatal Mortality	16	2.0
Cardiac/circulatory conditions	15	1.9
Hereditary and developmental abnormality	13	1.6
Integument/Musculoskeletal	9	1.1

Diseases of Sheep

In 2018, approximately 1368 ovine carcases were submitted for post-mortem examination. This comprised 798 lambs (from birth to one year of age) and 570 adult sheep (over one year of age).

The range of diagnoses varies according to age of the animal. Thus results in this section are presented by age category. In order to facilitate presentation and comparison, conditions which affect given systems have been grouped together.

> Vaccination for clostridial diseases remains an important part of on-farm disease control programmes.

Lambs (birth to 12 months of age)

Conditions affecting the digestive system were the most frequent diagnosis in lambs, with approximately 21 *per cent* of all lamb submissions being categorised thus. Within this category, parasitic gastroenteritis accounted for 44 *per cent* of diagnoses and infectious enteritis accounting for 43 *per cent*. *Strongyle spp.* and *Nematodirus spp.* were the most common parasites detected. Within the infectious enteritis category, there were a great variety of agents identified with *Coccidia spp.* and *Cryptosporidium spp.* being most commonly detected. Other conditions affecting the digestive system included; watery mouth, rumenitis and colitis.

The next most frequently diagnosed conditions were systemic conditions, affecting approximately 12 *per cent* of cases and respiratory conditions affecting approximately 11.8 *per cent* of cases.
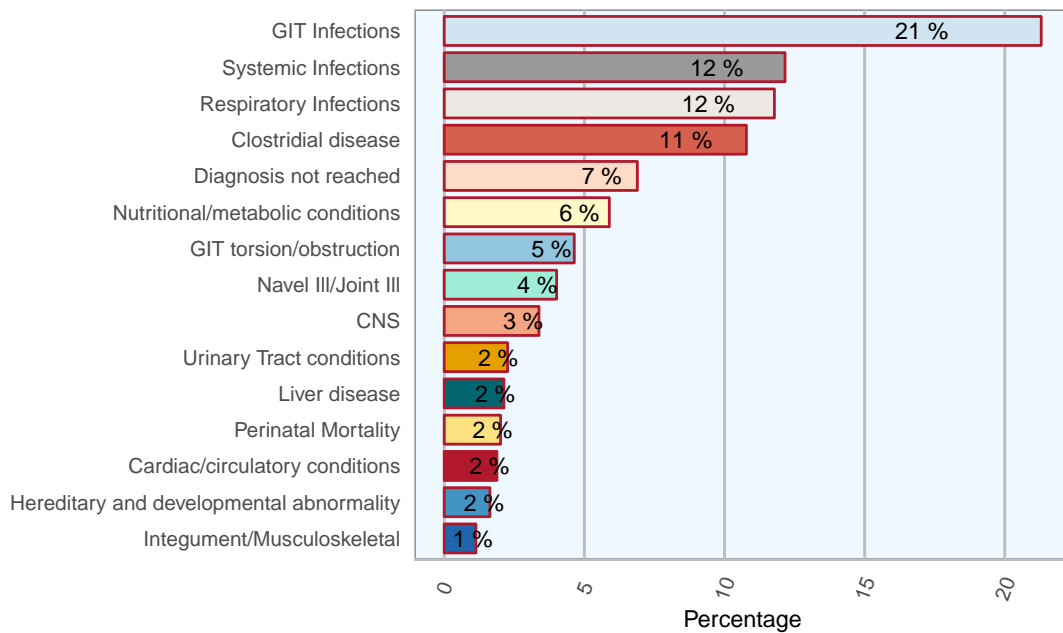
Within systemic conditions, bacteraemia/septicaemia, at 65 *per cent*, represents the largest diagnostic subcategory. Bacteraemia arises when bacterial infections succeed in overwhelming immune defences, is present in circulatory blood and can then result in embolic infection of multiple organs such as meninges, kidney,lung or liver. Septicaemia occurs where both pathogenic bacteria and their toxins are present in circulatory blood, toxins damage organs as they flow around the body resulting in toxaemia. Colloquially, this syndrome is known as *blood poisoning* and is characterised clinically by high temperature, shivering, weakness and inappetence. The most common agents identified in systemic infections were: *E. coli, Bibersteinia trehalosi* and *Mannheimia haemolytica*.

Figure 1: Conditions most frequently diagnosed on *post-mortem* examinations of lambs (from birth to one year of age) in 2018 (n= 798).

Figure 2: Lamb. *White-spotted* kidney: miliary 1–2 *mm* pus-filled white spots on renal cortical surface as a consequence of bacteraemia in a young lamb. *Staphylococcus aureus* isolated. Photo: Margaret Wilson.

Clostridial disease was diagnosed in one hundred and six cases. Eighty-six cases were in lambs, which are typically the most frequently affected age group. Pulpy kidney disease and enterotoxaemia were diagnosed most frequently. Both types of clostridial disease are associated with lamb stress and changes or increases of feed to lambs. Both result in rapid, often sudden, death and typically occur in well growing lambs. A breakdown of detected agents is presented in the *Bovine and Ovine Clostridial Diseases* section of this report.
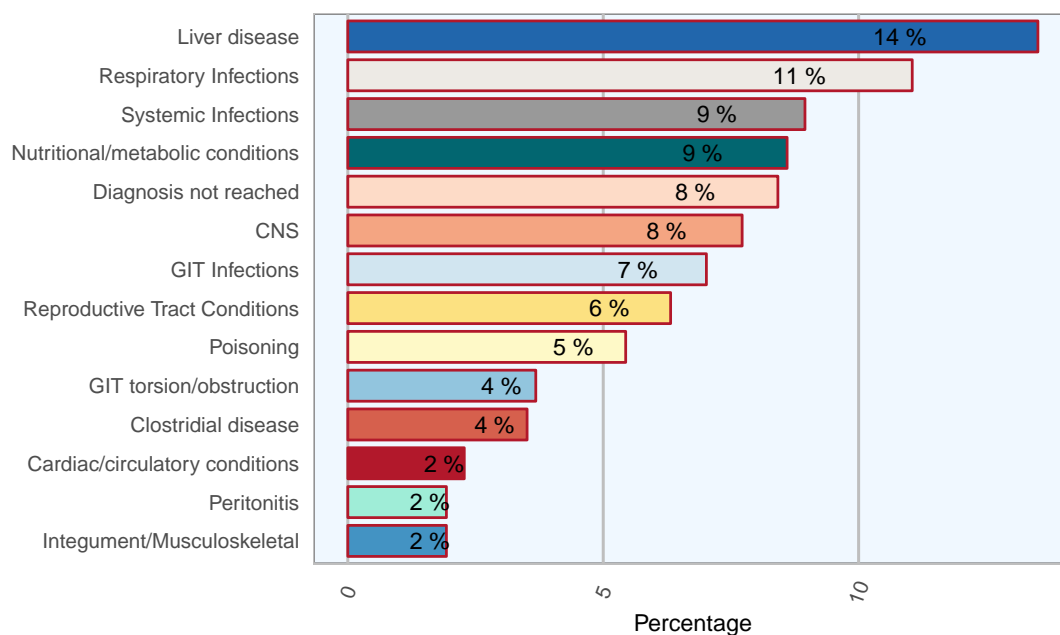
Thirty-two cases of navel ill/joint ill were detected in lambs. Navel ill/joint ill is associated with poor umbilical hygiene, low colostrum intake and high environmental contamination in lambing area, allowing bacterial introduction via umbilical stump within the first few hours of life. Infections may remain localised and develop into an umbilical abscess, extend locally to cause peritonitis, ascend to infect the liver or become generalised involving joints and/or other organs.

Adult Sheep (over 12 months of age)

Liver disease was the most frequent diagnosis in adult sheep with 77 cases, of which 74 were liver fluke (*Fasciola hepatica*). Liver fluke remains a common and serious threat to sheep health and welfare.

The next most frequent diagnosis was respiratory infection, of which 63 cases, 65 *percent*, were pneumonia. The most commonly detected pneumonic agents were; *Mannheimia haemolytica*, *Pasteurella multocida* and *Bibersteinia trehalosi*.

Six cases of Ovine Pulmonary Adenomatosis (OPA) were identified. OPA (Jaagsiekte) is a chronic incurable infectious lung tumor in sheep, caused by the Jaagsiekte sheep retrovirus. It has a long incubation

Figure 3: Conditions most frequently diagnosed (top 10 categories) on *post-mortem* examinations of adult sheep (over one year of age) in 2018 (n= 570).

Table 2: Conditions most frequently diagnosed on *post-mortem* examinations of adult sheep in 2018 (n= 570).

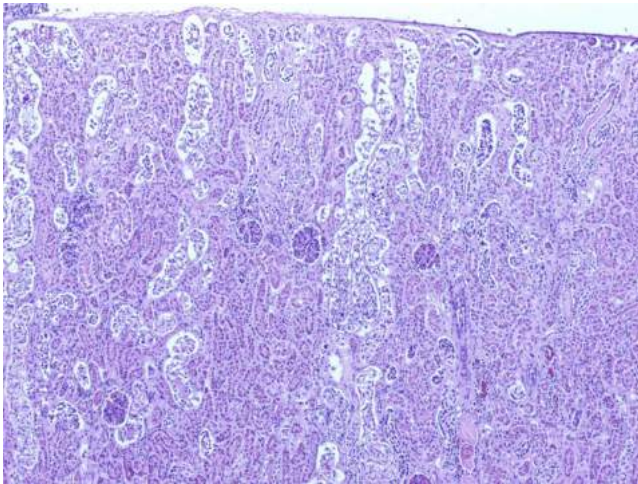| Category	No. of Cases	Percentage
Liver disease	77	**13.5**
Respiratory Infections	63	**11.1**
Systemic Infections	51	**8.9**
Nutritional/metabolic conditions	49	**8.6**
Diagnosis not reached	48	**8.4**
CNS	44	**7.7**
GIT Infections	40	**7.0**
Reproductive Tract Conditions	36	**6.3**
Poisoning	31	**5.4**
GIT torsion/obstruction	21	**3.7**
Clostridial disease	20	**3.5**
Cardiac/circulatory conditions	13	**2.3**

Figure 4: Sheep Lung: Severe diffuse fibrinosuppurative pleuropneumonia. *Pasteurella multocida* isolated. Photo: Margaret Wilson.

period, therefore, it is most commonly detected in adult sheep. Clinical signs are of progressive respiratory illness and weight loss. Secondary bacterial pneumonia is common and often the ultimate cause of death. Post mortem examination of lungs remains the only means of accurately diagnosing Ovine Pulmonary Adenomatosis (Lee *et al.*, 2017).

When it is necessary to carry out *post mortem* examinations in the field, it is essential that veterinary practitioners take the most appropriate samples for laboratory examination and also to preserve and package them properly. Pathologists at RVLs are available to give advice in this regard.

Central Nervous System diseases accounted for approximately 7.7 *per cemt* of adult sheep conditions diagnosed. Most commonly, CNS disease was attributed to Listeriosis (*Listeria monocytogenes*), based on bacterial isolation or presence of characteristic histological lesions in the brain.

Figure 5: Lamb Kidney: Pulpy Kidney Disease: Severe acute proximal tubule necrosis and tubular protein casts. *Clostrium perfringens* epsilon toxin detected. H&E 10x magnification. Photo: Margaret Wilson.

Thirty-one cases of poisoning were diagnosed in sheep, with copper toxicosis being most frequent. Sheep are predisposed to copper toxicity as the species, relative to other ruminants, has reduced capacity for copper excretion in bile. As a result, excess copper intake in sheep can be fatal.

References

Lee AM, Wolfe A, Cassidy JP, McV Messam LL, Moriarty JP, O'Neill R, Fahy C, Connaghan E, Cousens C, Dagleish MP, McElroy MC (2017). "First confirmation by PCR of Jaagsiekte sheep retrovirus in Ireland and prevalence of ovine pulmonary adenocarcinoma in adult sheep at slaughter." *Irish Veterinary Journal*, **70**(1), 33. ISSN 2046-0481. doi:10.1186/s13620-017-0111-z. URL https://doi.org/10.1186/s13620-017-0111-z.

Ovine Parasitic Disease

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Parasitic disease is consistently one of the most frequent *post-mortem* diagnosis in Regional Veterinary Laboratories (RVLs). In addition, faecal samples are submitted for clinical diagnosis; the majority of these samples come from herds or flocks with animals showing clinical signs of diarrhoea and/or weight loss. *Fasciola hepatica* (Fasciolosis),Trichostrongylidae (Parasitic Gastro-Enteritis) and *Dictocaulus viviparus* (Parasitic Pneumonia) are the most important organisms responsible for parasitic disease in cattle and sheep.

Agents of Parasitic Gastroenteritis

Faecal samples from young lambs are typically analysed for presence of trichostrongyle and nematodirus eggs, and coccidial and cryptosporidial oocysts. In older lambs (> 12 *weeks*) cryptosporidial oocysts is substituted for fluke eggs. Results generally specify whether liver and/or rumen fluke eggs are present but, in cases where a significant rumen fluke issue is suspected, testing of duodenal contents may be preferable.

The following report outlines trends in the results of faecal tests carried out at RVLs in 2018 and provides some interesting material for consideration. It is important to remember, however, that there can be a poor correlation between severe infestations and faecal egg counts. Extensive disease, and even death, can occur in the pre-patent period (time between larval infestation and appearance of eggs in the faeces) and factors other than parasitic burden may influence the number of eggs parasites produce.

For this reason, particularly where there have been unexpected deaths, additional submission of fresh carcases for post-mortem examination should always be considered.

Trichostrongylidae

In general, life cycles of the gastrointestinal nematodes (gut worms) are similar. Species of particular importance include *Teladorsagia circumcincta* (formerly *Ostertagia circumcincta*), *Trichostrongylus axei* and *Haemonchus contortus* which dwell in the abomasum, and *Trichostrongylus colubriformis* and *Cooperia curticei* which inhabit the small intestine.

These parasites are unable to multiply within sheep and their life cycle is direct (without need of an intermediate host). Pre-patent period is 16–21 *days*, depending on particular species and various environmental and host factors (Abbott K.A., 2012).

Traditionally, infestations would have been associated with storing lambs at autumn. However, in recent years problems have increasingly been seen in growing lambs from mid-summer onwards.
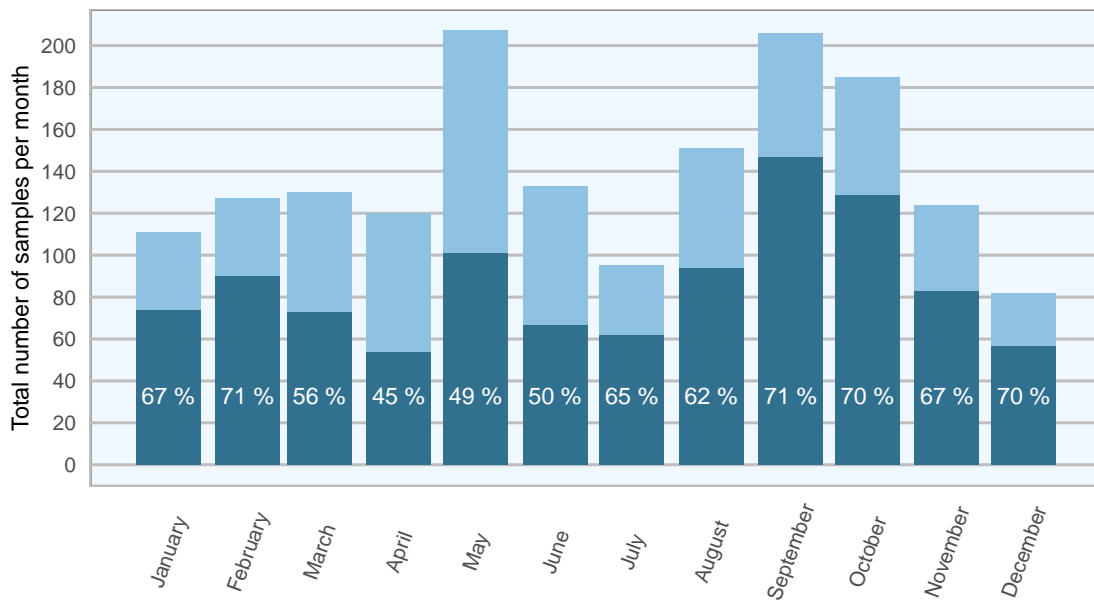
All the aforementioned species produce eggs of typical trichostrongyle appearance and are undistinguishable at this stage, consequently, they are analysed together.

In RVLs, Trichostrongyle egg counts tend to be substantially higher in ovine faecal samples than in bovine faecal samples (DAFM, 2016). There are many potential reasons for this though a greater focus on national ovine parasite control may be advisable.
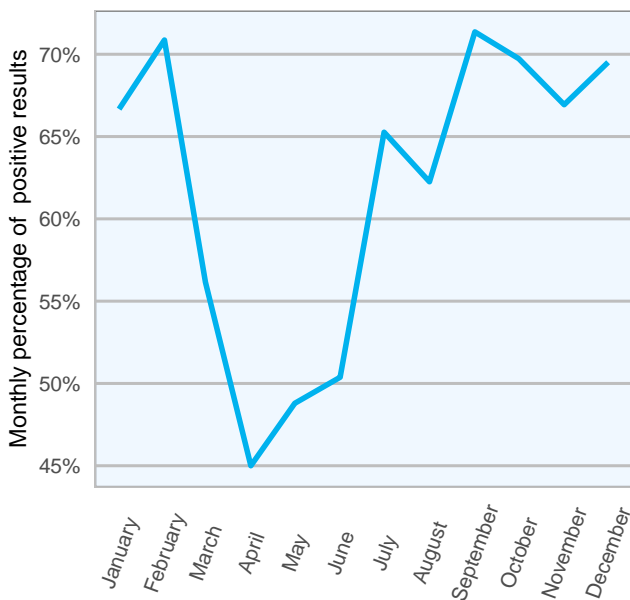
Table 1: Number of ovine faecal samples tested for *Trichostrongylidae* eggs in 2018 and results by percentage (n= 1673).

Result	No. of samples	Percentage
Negative	642	**38**
Low (50-500 epg)	499	**30**
High (>1200 epg)	301	**18**
Medium (500-1200 epg)	231	**14**

Of 1673 samples analysed in 2018 (Table 1) just under a third (32 *per cent*) were suggestive of significant (medium or high) trichostrongyle burdens. This suggests that, whilst the majority of animals appear to be bearing a "manageable" parasite load, there are proportionally still too many with potentially damaging levels of infestation, an increase of 5 *per cent* comparing 2018 RVL figures with 2016 All-Ireland ADSR (DAFM, 2016). In particular, the fact that there were more samples falling into the high burden category (18 *per cent*) than the medium one (14 *per cent*) is interesting as this does not reflect typical disease pattern. This may be suggestive of either issues with treatment (the possibility of anthelmintic resistance is always a concern) or else, perhaps, such results may indicate a bias towards testing only the most severely affected animals within the population, running the risk of overlooking those with less overt disease.

Figure 1: Stacked count of ovine faecal samples (all ages) tested per month for *Trichostrongylidae* during 2018. The percentage in each bar represents positive samples per month (n= 1673).

Figure 2: Percentage of positive ovine faecal samples for *Trichostrongylidae* eggs in 2018 (n= 1673).

The highest proportion of positive samples were examined in February and September (both 71 *per cent)*, indicating peaks at either end of the grazing season (Figures 1 and 2). The immunomodulatory effects of parturition are known to result in increased faecal parasitic shedding, which may reflect the first peak. The second increase might indicate build-up of parasite numbers in pasture as the season progresses. Samples were least likely to be positive between April and June, in keeping with heavy parasitic burdens taking a while to develop in growing lambs, particularly prior to weaning.

Sample numbers examined typically averaged at about 120 per month, with peaks of >200 examined in both May and September, and lesser numbers (<100) in both July and December. A greater number of sample submissions in Autumn would be expected, as this would typically be the time of year when most problems are seen, but reasons for the peak in May are a little less obvious.

Nematodirus

Nematodirus battus is another small intestinal nematode of importance in the Trichostrongyloidea superfamily, in this report it is considered separately due to differences in life cycle and identification.
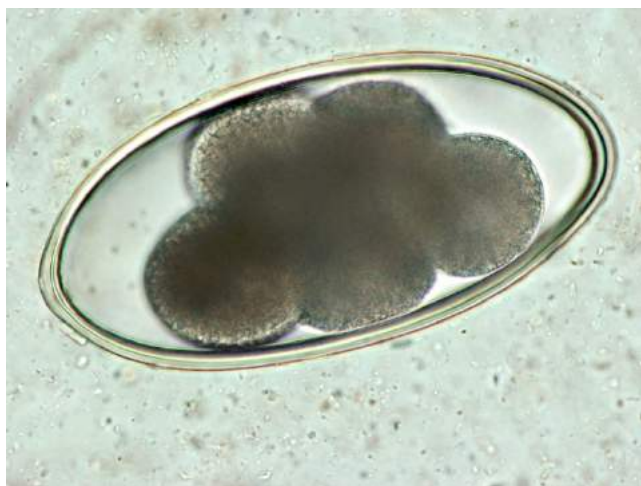
For all Nematodirus species, development as far as

L3 (the infective larval stage) occurs within the eggs after they are deposited on pasture, this development takes several months. In the spring, the synchronised mass hatch of overwintered infective larvae is usually triggered by a sudden increase in environmental temperature following a chilled period, a temperature differential greater than 10 °C is believed to be required for this phenomenon to occur (McMahon *et al.*, 2017).

As a result, many farmers have traditionally limited the impact of this disease by rotating grazing on an annual basis and by managing their stock carefully during the period of highest risk in spring. Although it has been shown that up to 70 *per cent* of eggs may be capable of hatching without being first stimulated by chill temperatures (van Dijk and Morgan, 2008) and, in recent years, patterns of disease appear to be changing, with many farms reporting a second smaller peak in clinical cases towards the end of the grazing season.

Nematodirosis typically affects growing lambs in their first season, usually at 6–12 *weeks* of age. Thankfully, immunity develops quickly and clinical disease is not an issue in adult stock.

Nematodirus *eggs* can be easily differentiated from typical trichostrongyle eggs; they are much larger, brownish in colour and have parallel sides (Figure 3).

Figure 3: Microphotograph of a *Nematodirus* egg. Photo: Cosme Sánchez-Miguel.

As the majority of damage with acute nematodirosis occurs in the pre-patent period and this parasite is known to be a relatively poor egg producer (DAFM, 2018), caution must be exercised when interpreting faecal egg count data. Accordingly, we note that although >85 *per cent* of faecal samples tested over the course of 2018 yielded negative results, this should not be considered evidence of freedom from infestation for the majority of those tested (Table 2).

Table 2: Number of *Nematodirus* eggs detected in ovine faecal samples in 2018 and results by percentage (n= 1673).

Result	No. of samples	Percentage
Negative	1431	**85.5**
Low (50-500 epg)	200	**12.0**
Medium (500-1200 epg)	29	**1.7**
High (>1200 epg)	13	**0.8**

Nonetheless, it is worth noting that thresholds dictating medium and high parasitic burdens are much lower for Nematodirus than for other gastrointestinal nematodes, >150 *epg* and >300 *epg* respectively. Detection of such burdens in faeces of lambs of a certain age are likely to be significant. Furthermore, presence of such burdens in animals which have recently received anthelmintic treatment should also raise concern as *Nematodirus battus* has traditionally shown very little evidence of resistance to Group 1 (Benzimidazole) anthelmintics, prior to the first case being reported in 2011 (Abbott K.A., 2012).
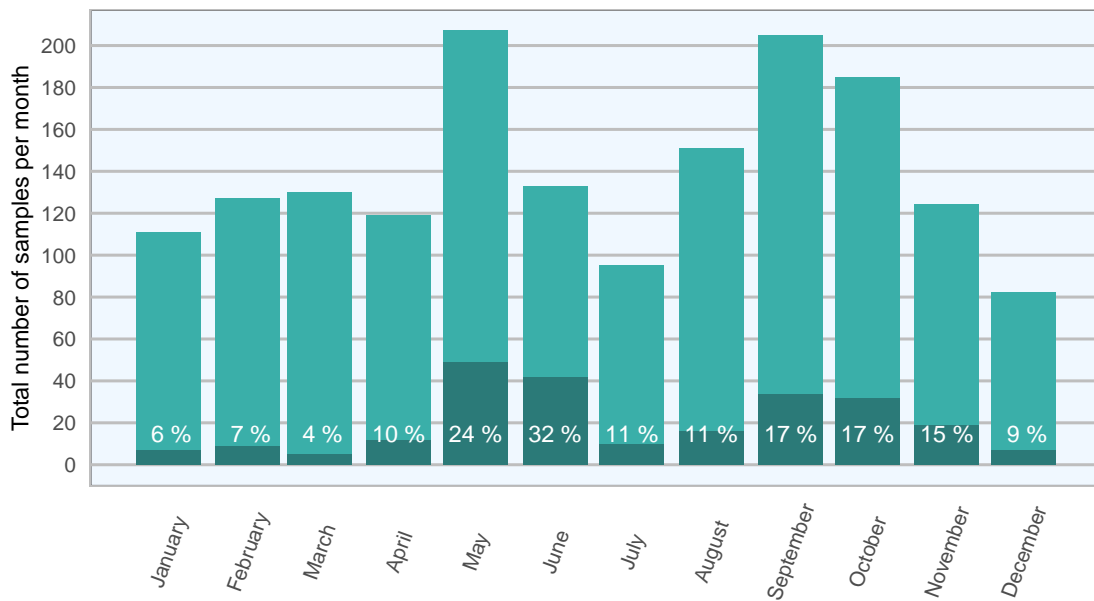
The lowest proportions of samples testing positive were observed from December to March; tying with expected seasonality and life cycle. The synchronised mass hatch at the end of April in 2018 (approximately two weeks later than usual due to a particularly cold spring) resulted in a small increase in April (Figures 4 and 5). Substantial increases were then seen in May and June, as higher numbers of parasites matured and commenced laying eggs. Finally, smaller increases observed in September and October may link in with a second, smaller, seasonal peak emerging in the annual disease pattern.

The largest numbers of samples were analysed in early summer and mid autumn, suggesting good awareness of when nematodirosis may be causing an issue.
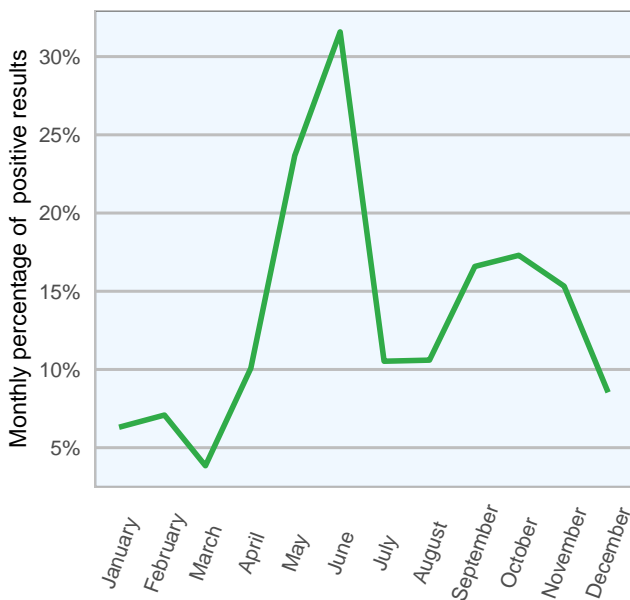
Coccidia

Coccidia are single celled (protozoan) parasites of the *Eimeria spp.* Species are host specific and, although there are some 15 different types that infect sheep, only two are believed to cause clinical disease; *Eimeria crandallis* and *Eimeria ovinoidalis*. Of these two, the latter is more pathogenic, causing extensive permanent damage to the lining of ileum, caecum and colon.

Disease tends to emerge from April/May onwards, primarily affecting 4–12 *week* old lambs with peaks at

Figure 4: Count of ovine faecal samples exmined for *Nematodirus* eggs in 2018. The percentage in each bar represents the number of positive samples per month (n= 1673).

Figure 5: Percentage of ovine faecal samples which tested positive for *Nematodirus* eggs in 2018 (n= 1673).
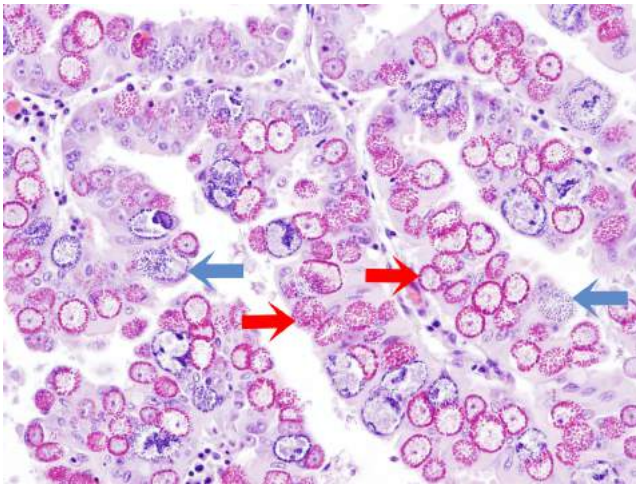
5–6 *weeks* when lambs first start to graze. Acute clinical disease is not commonly seen in individuals older than 3-4 *months* of age (Scott, 2012).

Ovine coccidian life cycle takes 12–20 *days* and differs to nematode life cycle in several ways; the most significant of these relates to the ability of coccidia to multiply internally by asexual and sexual reproduction (Figures 6 and 7), resulting in the number of oocysts shed being many millions higher than the number ingested, leading to very rapid levels of environmental contamination within very short periods.
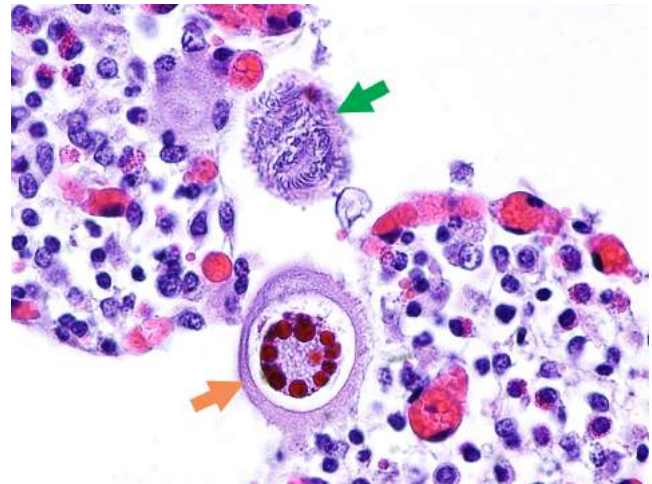
Table 3: Number of ovine faecal samples submitted in 2018 (all ages) for detection of coccidial oocysts and results by percentage, (n= 1693).

Result	No. of samples	Percentage
Not Detected	929	55
Light Infection	478	28
Moderate Infection	176	10
Heavy Infection	73	4
Severe Infection	37	2

Early in the season, the initial source of infection for lambs comes from the relatively small numbers of parasites shed by nursing ewes and oocysts which have survived over winter in the environment. The multiplier effect means that first infected lambs produce

Figure 6: Coccidial microgamonts (blue arrow) and macrogamonts (red arrow) in the intestinal villi of an sheep with severe coccidiosis. Photo: Cosme Sánchez-Miguel.

Figure 7: Coccidial microgamonts (green arrow) and macrogamonts (orange arrow) in the intestinal villi of an animal with severe coccidiosis. Photo: Cosme Sánchez-Miguel.

much higher levels of environmental contamination for lambs born later in the season.

Therefore, it is not unusual to see coccidiosis issues worsen over the course of the season, with lambs affected more severely and at a younger age, as the year progresses.

Disease tends to be self-limiting and immunity develops quickly, although there is no cross-protection between different species of coccidia (Scott, 2012). Often, survivors of clinical disease suffer permanent damage and fail to return to full health and acceptable growth rates despite successful treatment and management.

For this reason, preventative management is key. As coccidia are ubiquitous in the environment, complete avoidance of disease is considered impractical. Indeed, this may not even be advisable as some studies have shown that early exposure of young lambs to small numbers of parasites may be protective and, therefore, beneficial (Gregory, 1995).

As this tends to be an annual and recurrent issue, maintenance of good flock records can help to pinpoint particular risk periods allowing management and/or treatment protocols to be implemented at the most effective times in subsequent years (Scott, 2012).

Coccidial oocysts can be identified as pale, transparent, *egg-shaped* structures, with thin walls and many times smaller than typical trichostrongyle eggs. Large counts can be in excess of 100,000 oocysts *per gram*.

Unfortunately, as in the case of gastrointestinal nematode infestation, it must be remembered that faecal counts can be limited in several ways. Firstly, as

with nematode diseases, acute disease can occur before eggs or oocysts are observed in faeces. Secondly, as coccidial intestinal damage is cumulative, individual faecal oocyst counts may not be at their highest when clinical signs of disease are most pronounced. Furthermore, as the majority of species affecting sheep are not responsible for clinical disease, it is impossible to tell from oocyst counts whether pathogenic species are present and, if so, to what proportion of the count. This renders accurate interpretation of high counts impossible without requesting further testing to speciate the oocysts, which is not routinely conducted.

For the above reasons high faecal oocyst counts should always be interpreted with caution and in conjunction with clinical history and signs. It may be more useful to assess oocyst counts as indicators of general flock health rather than as a diagnostic tool for individual animals.

In 2018 (Table 3), 1693 ovine faecal samples were examined, 45 *per cent* were positive for coccidial oocysts and 16 *per cent* had counts suggestive of moderate to severe infestations. This is just over double the proportion of positive samples and almost treble the number of substantial counts than were seen with bovine samples examined during the same year. However, when comparing 2018 RVL figures with 2016 All-Ireland ADSR, there is a suggestion that proportion of positive samples examined has dropped by about 10 *per cent*.

In 2018 (Figures 8 and 9), the highest number of samples tested was in May (205) and the highest pro-

portion of samples testing positive was noted in June (65 *per cent*), both correlate well with the expected period of highest risk of clinical disease, when the infectious load has had a chance to build up and lambs are still vulnerable to disease, not yet having developed protective immunity. From February to May there is a progressively rapid increase in the proportion of positive samples examined, which may correlate with higher number of coccidia in environment due to repeated cycles of infection. After June, there is a general trend towards a lower proportion of positive results, presumably because immunity levels begin to rise from this point onwards. The lowest number of samples examined and the lowest proportion of samples yielding positive results occurred simultaneously in December, which again is logical, as this is the time of year where significant issues with coccidiosis would not be expected as there are few, if any, individuals of vulnerable age.
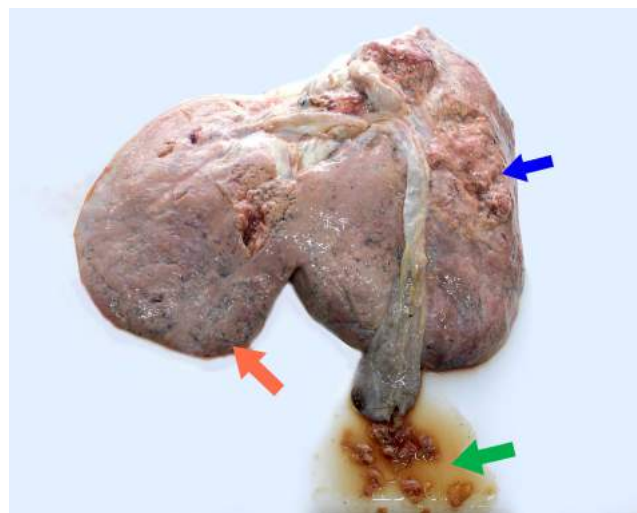
Liver and rumen fluke

Liver fluke disease in Ireland is associated with *Fasciola hepatica*, from the family *Fasciolidae*, that can infest livers of many mammals, including man. This flat leaf-shaped worm reaches an adult length of 25–30 *mm* and a width of about 13 *mm*, making it easily recognised during gross inspection of livers. There are two distinct forms of disease recognised; acute (Figure 10) and chronic. Both forms are not mutually exclusive and can be present in tandem within the same individual. In addition to the classic signs of disease outlined below, liver fluke has been shown to affect ewe fertility and milk yield, with consequential effects on flock economics (Hynes, 2010).

The most commonly identified rumen fluke of cattle and sheep in Ireland is *Calicophoron daubneyi*, from the family *Paramphistomidae*. Adults are usually 10 *mm* in length, pink, fleshy, tear-drop shaped and occupy ruminal and reticular surfaces. Rumen fluke infestations have been an increasingly common finding in Irish ruminants over the past decade or so (Manual, 2013).

For many years rumen fluke was believed to be of negative or very limited pathogenicity but, over recent years, studies have associated high burdens of rumen fluke with outbreaks of profuse watery foetid diarrhoea, dullness, inappetence and even sudden death in both cattle and sheep, typically in immature stock (Kajugu *et al.*, 2015). This particularly seems to be the case when very high numbers of immature stages are present in the small intestine (principally the duodeunum) without necessarily featuring presence of adults in rumen, nor eggs in faeces. Knowledge of the entire life cycle and epidemiology of rumen fluke is still limited and presence of adult parasites is not believed to be associated with clinical disease.
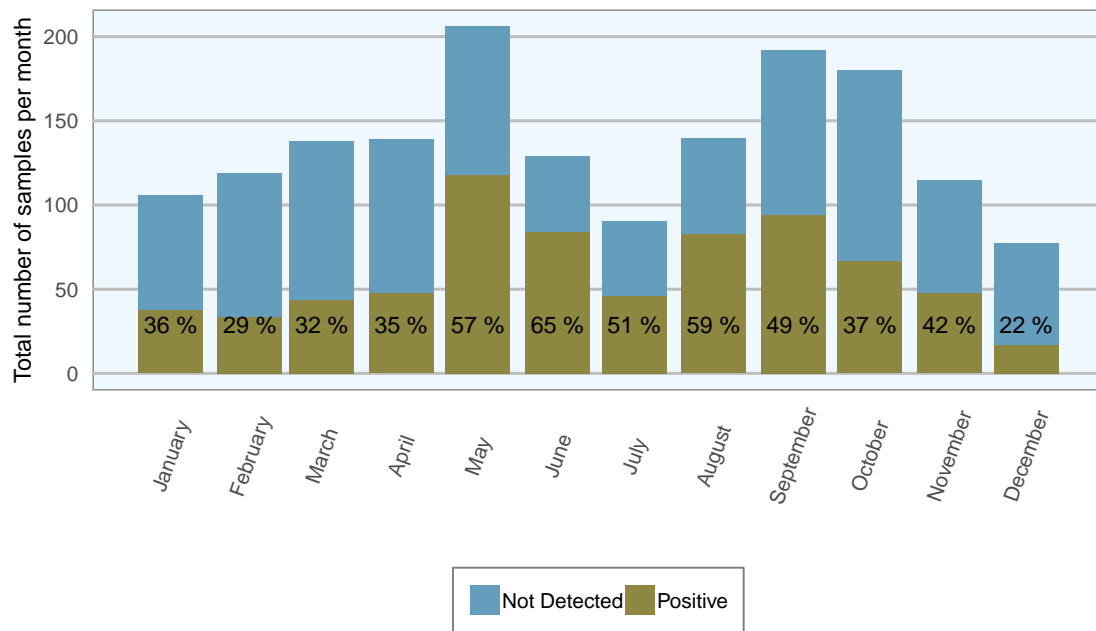

Figure 10: Photograph demonstrating characteristic lesions associated with acute faciolosis in the liver of a lamb. Note friable liver (blue arrow), subcapsular haemorrhagic tracts (orange arrow) and trematodes of *Fasciola hepatica* inside the gallbladder (green arrow). Photo: Cosme Sánchez-Miguel.

The fluke life cycle is protracted with environmental development taking anything from five weeks to three months, depending on underlying weather and temperature conditions. Temperatures greater than 10 °C are required for fluke eggs to hatch and for the mud snail intermediate host (*Galba truncatula*) to become active. Pre-patent period, from infection to commencement of egg laying, usually takes 9–12 *weeks*.
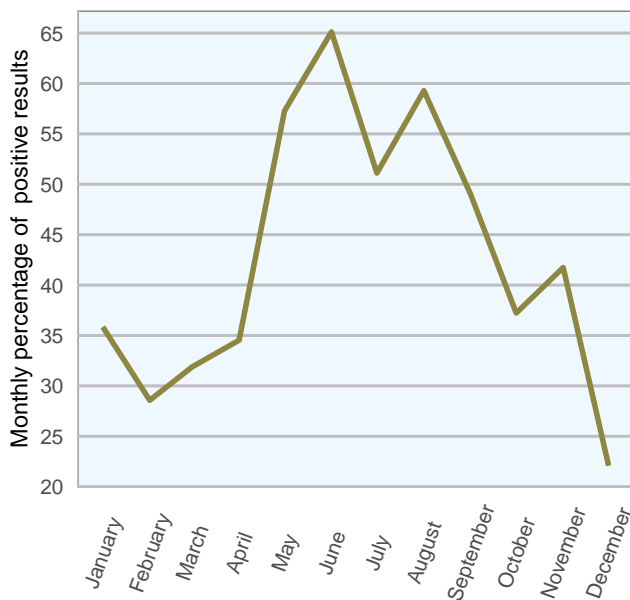
Table 4: Number of ovine faecal samples submitted in 2018 (all ages) for detection of liver fluke eggs and results by the percentage (n= 1441).

Result	No. of samples	Percentage
Liver fluke eggs not detected	1301	90
Positive liver fluke eggs	140	10

Classically, acute liver fluke cases would be first seen in mid-late autumn, with chronic cases emerging in winter over the housing period. However, as with many parasitic infestations, traditional epidemiology of liver fluke has changed over recent years. Perhaps climatic pattern fluctuations over the past decade, with milder

Figure 8: Stacked number of ovine faecal samples (all ages) tested for coccidial oocysts in 2018. The percentage in each bar represents the number of positives per month (n= 1693).

Figure 9: Count of ovine faecal samples examined for coccidial oocysts in 2018 (n= 1693).

winters and warmer wetter summers, have encouraged the emergence of new epidemiological variations.

Sheep tend to be more severely affected by liver fluke than cattle, particularly by acute disease. There is little evidence of age related or developmental immunity to liver fluke in either species, meaning adult livestock should be considered equally at risk as youngstock. Presence of liver and rumen fluke can be confirmed by identification of immature or adult stages at post-mortem or by faecal examination to identify presence of eggs. Eggs are of similar appearance but, whilst liver fluke eggs are of a consistent shape with a yellow or gold hue, rumen fluke eggs tend to be more variable in shape and clear in colour.

Presence of fluke eggs in faeces is indicative of presence of adult fluke within bile ducts, and a reasonable indicator for chronic liver fluke disease. However, it is not reliable for identifying cases of disease associated with rumen fluke, nor acute liver fluke disease; for both conditions damage takes place exclusively within the pre-patent period. Furthermore, caution should be taken when interpreting faecal egg counts, as fluke egg counts tend to be low and generally provide a less reliable indicator of overall parasitic burden than most nematode species. In addition, presence of rumen fluke eggs in faeces may not be indicative of disease at all. Therefore, it is again worth emphasising the value of

Figure 11: Microphotograph of rumen fluke (*Calcicophoron daubneyi*) in the small intestine. Photo: Seamus Fagan.

post-mortem examination and abattoir inspections in conjunction with faecal egg count results when investigating suspected fluke outbreaks.

In 2018, 10 *per cent* of 1441 ovine faecal samples examined were positive for liver fluke eggs and 20 *per cent* were positive for rumen fluke eggs. Once again,comparing 2018 RVL figures with 2016 All-Ireland ADSR (DAFM, 2016), this is an improvement from the 2016 figures (14 *per cent* and 23 *per cent* respectively). It should be noted that, in 2018, a colder than usual winter/spring followed by an unusually dry summer is perhaps likely to have exerted more of an influence than changes in disease management and control.

In 2018, the highest proportion of ovine samples testing positive for liver fluke eggs was observed in June (67 *per cent*), replicating trends towards detecting chronic disease earlier in the year. The lowest proportion of samples testing positive was in April (17 *per cent*), perhaps not unexpected given the unseasonably cold and icy conditions that prevailed in March/April last year. The largest numbers of samples tested was in October, probably reflecting testing with a view to deciding merits of administering anthelmintic treatment prior to housing. The lowest number of samples tested was in June, although the fact that this coincided with the highest proportion of positive samples suggests that complacency with regard to sampling for liver fluke in

mid-summer may be inadvisable from now on.

In 2018, the highest proportion of ovine samples testing positive for rumen fluke eggs was in October (98 *per cent*) and the lowest in June (53 *per cent*). These fluctuations may correlate to as yet unestablished elements of the rumen fluke life cycle and seasonality but, as little is currently known about the significance (if any) of chronic rumen fluke infestation in sheep or cattle in Ireland, it is probably not worth commenting further. However, it could be noted that these results suggest that infestation with mature rumen fluke is a common finding in the majority of ovine faecal samples examined, regardless of time of year.

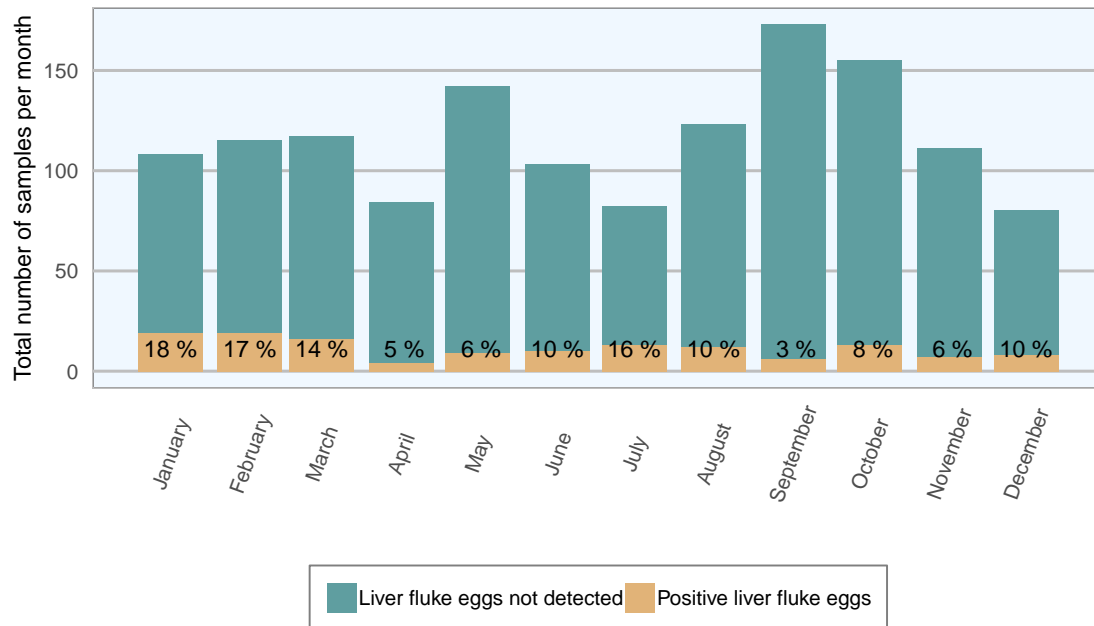**Table 5:** Presence of rumen fluke eggs in ovine faecal samples in 2018 (n= 1441).

Result	No. of samples	Percentage
Rumen fluke eggs not detected	1155	**80**
Positive rumen fluke eggs	286	**20**

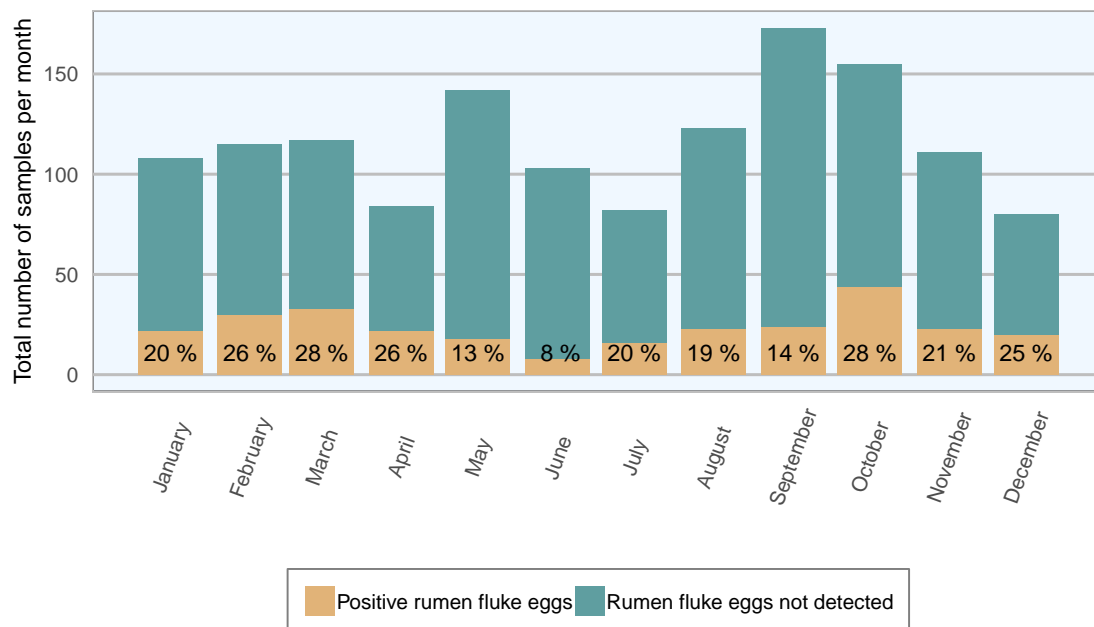## Sarcocystosis & Cysticercosis

In an addition to typical ovine parasitic diseases regularly discussed in this report, a combined outbreak of ovine sarcocystosis and cysticercosis came to light in March 2018 as a result of lesions observed at abattoir slaughter.

The increased presence of *cyst or pimple-like* lesions in lamb carcases from an intensive indoor lamb finishing unit, leading to excessive muscle trimming and carcase condemnations, raised initial concerns. Further laboratory testing involving histopathological and multiplex PCR technology confirmed the involvement of both *Taenia ovis* (tapeworm) and *Sarcocystis tenella* (protozoan) (O'Shaughnessy, unpublished data, 2019).
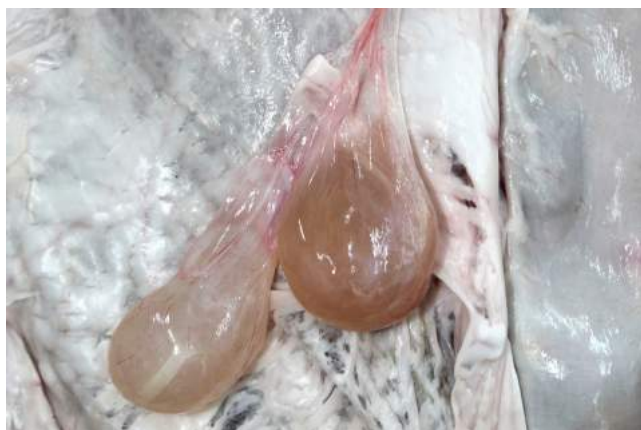
Both parasites utilise dogs as definitive hosts and small ruminants as intermediate hosts. Hosts are seldom significantly impacted clinically by the presence of either parasite. However, sarcocystosis has occasionally been shown to result in reduced flock performance and sporadic ovine abortions (Dubey *et al.*, 2015).

Figure 12: Stacked number of ovine faecal samples (all ages) tested for liver fluke in 2018. The percentage in each bar represents the number of positive samples per month (n= 1441).

Figure 13: Stacked count of ovine faecal samples (all ages) tested for rumen fluke. The percentage in each bar represents positive samples per month (n= 1441).

Figure 14: *Cysticercus tenuicollis* in the peritoneal cavity of a sheep. Photo: Cosme Sánchez-Miguel.

In the case of the 2018 outbreak, the scale of carcase condemnation resulted in economic and food safety implications that had to be addressed. On farm investigations revealed risk factors such as suboptimal anthelmintic dosing of farm dogs, inappropriate storage of ovine carcases and carnivore access to feed stores. In addition, excessive pruritus, due to *Psoroptes ovis* (*sheep scab*), and lameness were also identified; these had not previously come to light and were identified during farm visits.

This case clearly highlights that indoor lamb finishing units, which are a novel adaptation of sheep farming in Ireland, may be subject to variations in disease prevalence and presentation and should invest in robust flock-health planning with ongoing veterinary oversight to ensure maintenance of acceptable flock health and welfare levels.

References

Abbott KA Taylor M SL (2012). "SCOPS Technical Manual." URL https://www.scops.org.uk/workspace/pdfs/scops-technical-manual-4th-edition-updated-september-2013.pdf.

DAFM (2016). "All-island Animal Disease Surveillance Report." URL https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/labservice/rvlreports/AIDSRReport016230118.pdf.

DAFM (2018). "2018 Nematodirus Forecast." URL https://www.agriculture.gov.ie/press/pressreleases/2018/april/title,116630,en.html.

Dubey J, Calero-Bernal R, Rosenthal B, Speer C, Fayer R (2015). *Sarcocystosis of Animals and Humans*. Second edition. edition. CRC Press. ISBN 9781498710152 1498710158.

Gregory MW (1995). "Prevention of coccidiosis in lambs." *In Practice*, **17**(8), 382–382. ISSN 0263-841X. doi:10.1136/inpract.17.8.382-a. https://inpractice.bmj.com/content/17/8/382.2.full.pdf, URL https://inpractice.bmj.com/content/17/8/382.2.

Hynes F (2010). "Internal Parasites: An Ongoing Problem on Sheep Farms." URL https://www.teagasc.ie/media/website/publications/2010/InternalParasites.pdf.

Kajugu P, Hanna R, Edgar H, McMahon C, Cooper M, Gordon A, Barley J, Malone F, Brennan GPand Fairweather I (2015). "Fasciola hepatica: Specificity of a coproantigen ELISA test for diagnosis of fasciolosis in faecal samples from cattle and sheep concurrently infected with gastrointestinal nematodes, coccidians and/or rumen flukes (paramphistomes), under field conditions." *Veterinary Parasitology*, **212**(3), 181 – 187. ISSN 0304-4017. doi:https://doi.org/10.1016/j.vetpar.2015.07.018. URL http://www.sciencedirect.com/science/article/pii/S0304401715003477.

Manual CT (2013). "Control of Liver and Rumen Fluke in Cattle." URL https://www.cattleparasites.org.uk/app/uploads/2018/04/Control-liver-and-rumen-fluke-in-cattle.pdf.

McMahon C, Edgar HWJ, Barley JP, Hanna REB, Brennan GP, Fairweather I (2017). "Control of Nematodirus spp. infection by sheep flock owners in Northern Ireland." *Irish Veterinary Journal*, **70**(1), 31. ISSN 2046-0481. doi:10.1186/s13620-017-0109-6. URL https://doi.org/10.1186/s13620-017-0109-6.

Scott P (2012). "Coccidiosis in Sheep." URL https://www.nationalsheep.org.uk/workspace/news-pdfs/12-03-Coccidiosis-in-Sheep(E)29032012122058.pdf.

van Dijk J, Morgan E (2008). "The influence of temperature on the development, hatching and survival of Nematodirus battus larvae." *Parasitology*, **135**(2), 269–283. doi:10.1017/S0031182007003812. URL https://www.cambridge.org/core/journals/parasitology/article/influence-of-temperature-on-the-development-hatching-and-survival-of-nematodirus-battus F7BDAC5757170E82B5C49FA0423C516F.

Diseases of Pigs, Poultry and Deer

Veterinary Laboratory Service

2018

Animal Disease Surveillance Report

Diseases of Pigs

Shane McGettrick[a]

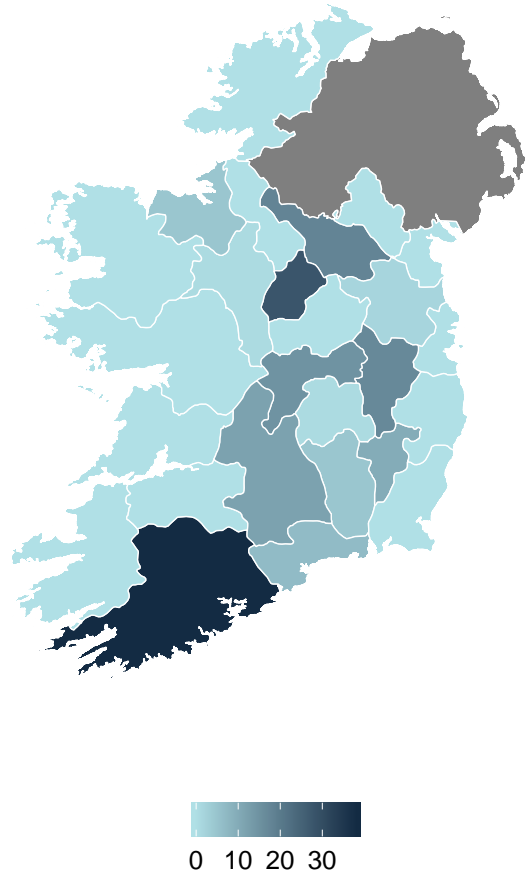[a]Senior Research Officer, Sligo Regional Veterinary Laboratory, Doonally, Sligo, Ireland

The Irish pig industry comprises 1.7 million pigs in approximately 1674 active herds. Two-thirds of these herds are made up by small scale producers who have less than six pigs, while there are 271 herds with greater than 100 pigs. The larger herds are intensively managed highly integrated systems whose private veterinary needs are provided by a relatively small group of specialist pig veterinarians. DAFM laboratories are attempting to increase the laboratory support to the pig industry through the provision of specialist laboratory expertise and continuing engagement with pig veterinary consultants and advisors. A primary focus has been surveillance for new and emerging diseases combined with an emphasis on the role of biosecurity in reducing risk of disease incursion and lessening impact of endemic disease in pig herds.

In 2018, DAFM laboratories carried out necropsy examinations on 164 pig carcases, while non-carcase diagnostic samples were submitted from 3201 pigs for various tests to assist veterinarians with disease investigation and/or surveillance on Irish pig farms. Figures 1 and 2 illustrate the number of pig carcases and diagnostic samples submitted from each county in 2018. The Irish pig population is not distributed evenly throughout the country; this is reflected in submission rates to laboratories, with the highest number of samples coming from counties with the highest pig populations.

Post mortem diagnoses

The most frequent diagnoses in pig necropsy submissions during 2018 are detailed below. It should be understood that these reflect diagnoses in animals submitted to DAFM laboratories, rather than incidence of disease in the pig population, as many factors will influence the decision to submit an animal for necropsy.

Gastrointestinal disease. Enteritis was the most frequent diagnosis in pig carcases submitted during 2018, the majority of these were in suckling pigs. Neonatal diarrhoea is a significant cause of morbidity and economic loss on Irish farms and necessitates the use of increased levels of antibiotics to reduce the impact of disease in the highly vulnerable intensive production systems. Neonatal piglets have reduced capacity to produce hypertonic urine, meaning that they are very susceptible to dehydration if the absorptive capacity of the gut is damaged. There are a wide number
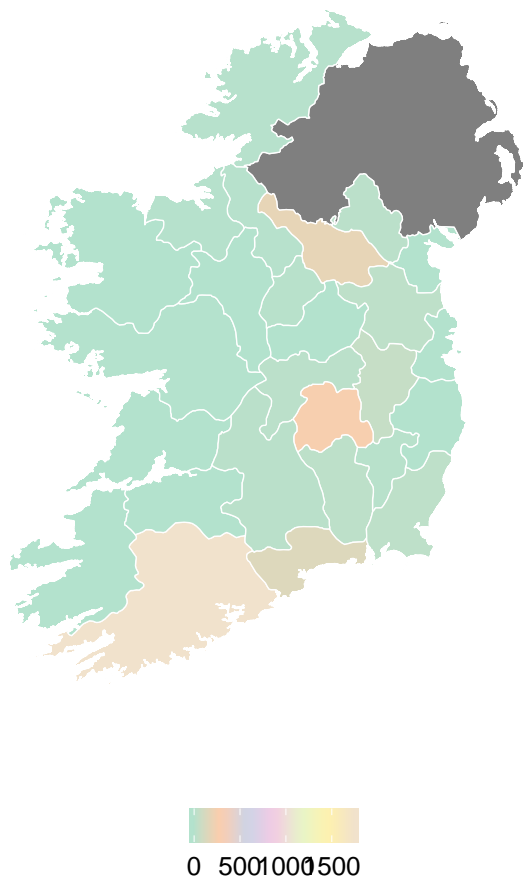

Figure 1: Number of carcass per county submitted to the RVLs for *post-mortem* examination during 2018.

of agents implicated in neonatal pig diarrhoea; most common infectious agents identified in nursing pigs with diarrhoea are *Clostridium perfringens*, Rotavirus, *E.coli*, *Cryptosporidia spp.*, *Isospora suis* and *Clostridium difficile*.

Porcine epidemic diarrhoea (PED), that caused devastating disease in the US during 2014, has not been detected in Ireland but is present in some European countries. Hence, disease surveillance in pigs showing enteric disease is ever more important. In undiagnosed outbreaks of diarrhoea, veterinary practitioners are encouraged to contact DAFM laboratories to avail of support and advice on sampling and diagnostic investi-

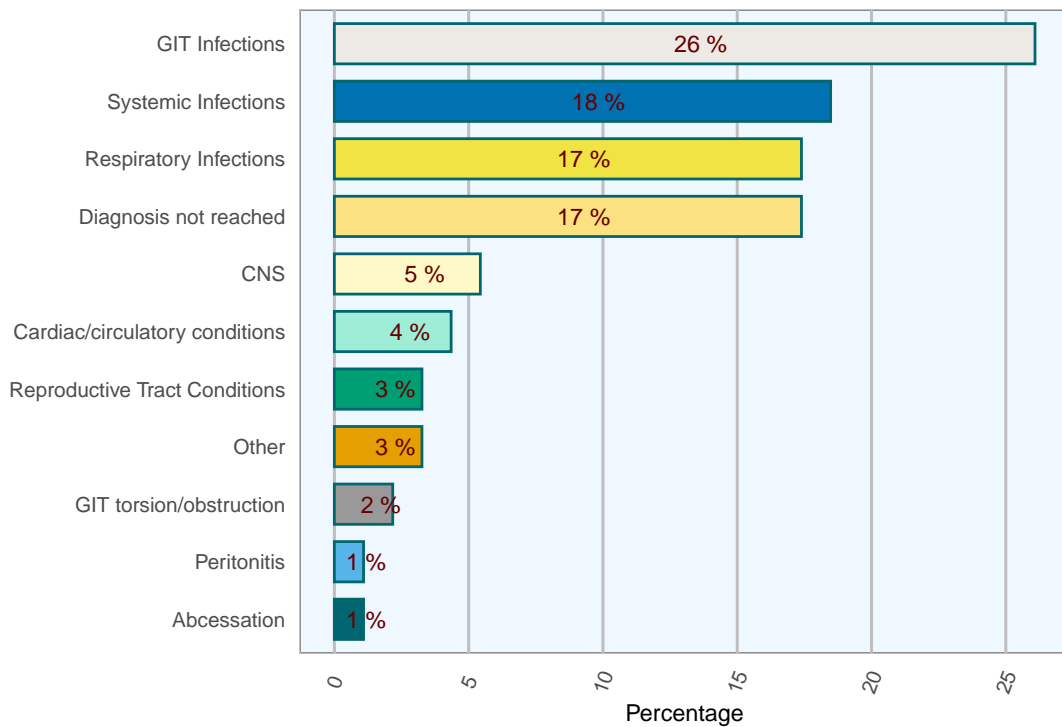gations, including detailed necropsy and histopathology.

damage to agricultural industry and decreased animal welfare. Absence of definitive aetiological diagnosis and misunderstanding of the relative contribution of various pathogens to disease pathogenesis and subsequent clinical signs may lead to the use of inappropriate treatments, particularly antibiotics, which is likely to contribute to development of antibiotic resistance.

0 500 1000 1500

Figure 2: Number of non-carcass diagnostic samples per county submitted to the RVLs during 2018.

Table 1: Diseases diagnosed in pigs submitted for *post-mortem* examination to the RVLs in 2018 (n= 92).

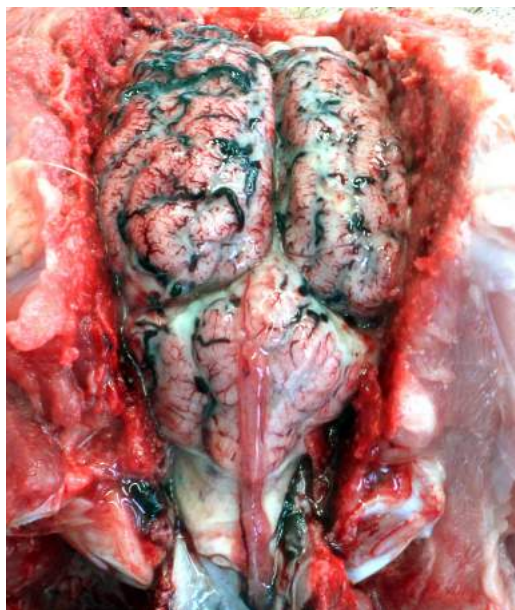| Diagnosis	No. of cases	Percentage
GIT Infections	24	26.1
Systemic Infections	17	18.5
Diagnosis not reached	16	17.4
Respiratory Infections	16	17.4
CNS	5	5.4
Cardiac/circulatory conditions	4	4.3
Other	3	3.3
Reproductive Tract Conditions	3	3.3
GIT torsion/obstruction	2	2.2
Abcessation	1	1.1
Peritonitis	1	1.1

Enteric disease is by far the most prevalent and significant health problem affecting neonatal pigs worldwide. Recent diagnostic investigations in Pathology and Virology Division in the Central Veterinary Research Laboratory (CVRL) and the emergence of so-called New Neonatal Porcine Diarrhoea Syndrome in Europe have shown that, in many cases, the aetiology of neonatal pig diarrhea is multifactorial and often cannot be attributed solely to the main pathogens traditionally associated with diarrhoea. It is likely that the relatively low diagnostic rate in these cases is due to a mixture of inadequate availability of diagnostic tests, emergence of novel agents, multi-pathogen infections, complex interactions between gut microbiome and environmental stresses. Inability to determine the underlying cause has implications in terms of control, antibiotic treatment, economic costs to the farmer, reputational

Systemic disease. Rapidly growing pigs in weaner and finisher stages are susceptible to various bacterial diseases which are usually opportunistic infections that become established due to dietary change or other environmental stressors. *Streptoccus suis* and oedema disease due to toxin-producing *E. Coli* were frequent causes of systemic disease in pigs in 2018.

Figure 3: Diseases diagnosed in pigs submitted for *post-mortem* examination to the RVLs in 2018 (n= 92).

Figure 4: Fibrinosuppurative meningitis. Cranial view. Pale yellow-white exudate in the subarachnoid space, composed of neutrophils, fibrin and bacteria, and caused by *Streptococcus suis* type 2. Photo: Shane McGettrick.

Streptococcus suis. Most pigs carry *Streptococcus suis* in the upper respiratory tract, piglets are likely to have been infected by their mothers. However, few carry the virulent strains capable of causing disease. An outbreak of disease is likely to occur when subclinical carriers of virulent strains are introduced into a population; this was considered the most likely method of transmission in cases described below where management systems included mixing and movement of pigs between groups. The severity of disease, even when the virulent strain is present, is variable and thought to be dependent on the presence of other stress factors including concurrent disease, high stocking density, environmental stress and recent mixing. Cases described below were considered relatively unusual in that the presentation was acute rather than the more insidious onset of disease expected. Environmental and management factors were likely to have been significant factors in the occurrence of disease.

In 2018 DAFM laboratories investigated various increased mortality events in weaner and finisher pigs in large scale commercial pig units. In one such case, clinical history was of pigs weighing 15–30 *Kg* dying suddenly without any observed illness. No management change was identified but severity was considered worst in younger pigs and disease developed within two weeks of entering the second stage weaner facilities. The unit did not operate an *all-in-all-out* policy
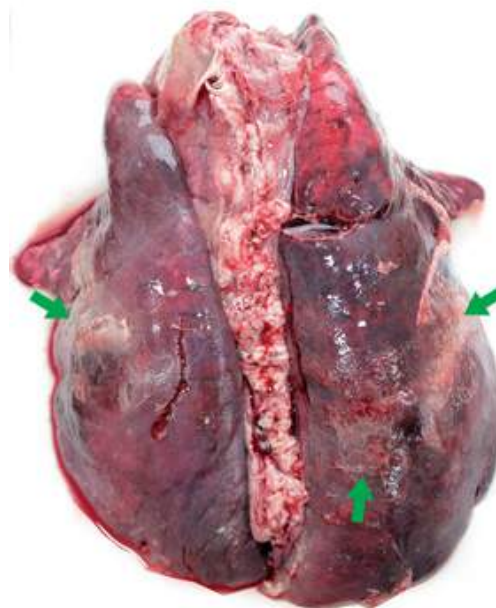
in weaner and fattener houses; instead it batched pigs on basis of weight. Pigs submitted for necropsy were in good condition although there was marked reddening of ventral neck and abdomen noted in all cases. Necropsy revealed similar findings in all pigs examined; variable interlobular pulmonary oedema and excess straw coloured fibrinous pericardial, pleural and abdominal fluids. The meninges of all pigs were considered cloudy on gross examination (Figure 4) and an acute fibrinosuppurative meningitis was confirmed by histopathology. *Streptococcus suis* was isolated in all pigs from multiple organs including brain.

Streptococcus suis was identified as the cause of severe acute polyarthritis in early stage fattener pigs in another farm. Clinical signs included sudden increase in lameness and ill thrift. Necropsy identified severe acute fibrinosuppurrative arthritis affecting multiple joints with associated soft tissue oedema and swelling. Diagnosis was confirmed by pure growth cultures of streptococcus suis from joint material.

Respiratory disease. Respiratory disease in pigs is a cause of ongoing production loss in many pig units. In 2018, DAFM laboratories continued its ongoing collaboration with research partners in investigating causes of respiratory disease on Irish pig farms. Results from this work are being disseminated to the industry.

An example of a respiratory disease case investigation during 2018 was where a number of pigs were submitted following a rapid increase in mortality in a large commercial pig herd. Late stage weaners and early stage fatteners were reported as dying following acute respiratory illness. Marked reddening of ventral body areas was reported consistently and the farmer recorded 1–2 pigs dead or dying each morning in affected batches. Six pigs submitted for necropsy were diagnosed with pleuritis. There was severe diffuse fibrinous pleuritis and pericarditis with large amounts of fresh fibrin deposited on thoracic serosal surfaces. Pulmonary lesions were considered extremely severe with diffuse consolidation of 80–90 *per cent* of lung parenchyma and marked necrosis of parenchyma in diaphragmatic lobes in all pigs. There was mild excess of abdominal fluid and small amounts of fibrin in some pigs. Other serosal surfaces, including joints and meninges, were unremarkable. Distribution and severity of lesions were consistent with *Actinobacillus pleuropneumoniae* (APP) infection, which was later confirmed by culture and histopathology.

APP is a relatively commonly diagnosed cause of

Figure 5: Porcine pleuroneumonia. Fibrinous bronchopneumonia caused by *Actinobacillus pleuropneumoniae*, characterised by severe pulmonary consolidation and fibrinous exudate on pleural surface (green arrows) mainly affecting the caudal lobes. Photo: Cosme Sánchez-Miguel.

pneumonia in pigs (Figure 5). Severe outbreaks may occur in naive populations causing a rapidly fatal pleuropneumonia. Source of infection is likely to be previously infected pigs where organisms remain as *sequestra* in pulmonary parenchyma or in tonsils and nasal cavity. There is marked differences in virulence of strains and disease is difficult to eradicate from herds. Severity of the disease is dependent both on the virulence of the train and the infectious dose. In experimental conditions death has occurred as rapidly as 3 *hours* post inoculation.

Notifiable disease

African swine fever awareness. The current outbreak of African swine fever (ASF) in Europe poses a risk to the Irish pig industry (Sánchez-Cordón *et al.*, 2018). The disease, which has been increasingly identified over the past year in wild boar and commercial pig farms in Eastern Europe, was identified in wild boar populations in southern Belgium in 2018. The most significant risk factor for entry into Ireland is feeding illegally imported

infected pork products to pigs. During 2018, DAFM veterinary laboratory service placed a strong emphasis on preparation and contingency planning to mitigate risk from a potential incursion of exotic disease such as ASF through increased training of staff on outbreak investigations and pig sampling techniques.

ASF is a notifiable disease and PVPs are reminded to notify DAFM if they suspect presence of the disease by contacting their local RVO or the National Disease Emergency Hotline at 1850 200 456. An ASF factsheet for vets detailing the clinical signs of ASF is available on the African swine fever page on the DAFM website. DAFM also produced a biosecurity leaflet specifically aimed at non-intensive pig farms and an ASF factsheet for farmers, both are available to download from the African swine fever page on the website.

Non-intensive or smaller pig herds as well as pet pig owners, may have irregular veterinary input and are likely to contact their local veterinary practitioner for advice when faced with unexplained clinical signs or deaths. DAFM laboratories are aware of the difficulties in reaching a diagnosis in these cases, especially for veterinary practitioners who may not have previous experience in treating or diagnosing the range of disease that may be present in pigs. All practitioners are reminded that, in any relevant pig disease outbreak, DAFM laboratories are available to offer advice on sampling and will carry out necessary testing, including necropsy free of charge, in order to confirm a diagnosis.

Practitioners are also advised to encourage clients with small pig herds to submit any dead or fallen carcases to the DAFM laboratory network, as this will provide valuable disease surveillance material and will allow the submitting vet to assist in diagnosis and management of disease within the herd.

References

Sánchez-Cordón P, Montoya M, Reis A, Dixon L (2018). "African swine fever: A re-emerging viral disease threatening the global pig industry." *The Veterinary Journal*, **233**, 41 – 48. ISSN 1090-0233. doi: https://doi.org/10.1016/j.tvjl.2017.12.025. URL http://www.sciencedirect.com/science/article/pii/S109002331730268X.

Poultry Diseases

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Avian Influenza Surveillance

Avian influenza type A is a contagious disease caused by viruses which are naturally found in, and adapted to, populations of wild birds. Avian influenza viruses can also affect poultry and mammalian species including rodents, pigs, cats, dogs, horses and humans.

Based on the severity of the disease, Avian Influenza is divided into low pathogenic (LPAI) and high pathogenic (HPAI) strains. LPAI may present with mild or no clinical signs in poultry. On the other hand, HPAI strains can cause severe clinical signs such as respiratory signs, reduced food intake, diarrhoea and nervous signs. In some cases, HPAI strains can cause sudden death with no other symptoms; in layers, drop in egg production and poor egg quality has been reported.

Avian Influenza viruses are classified into subtypes based on two surface proteins: haemagglutinin (HA) and neuraminidase (NA). H5 and H7 subtypes have been associated with acute clinical disease in chickens, turkeys and other birds of economic importance.

Active surveillance. DAFM carries out two types of active surveillance for avian influenza.

Serology testing in poultry for the national Poultry Health Programme (PHP). The Poultry Health Programme is a DAFM surveillance programme supporting poultry trade and complying with EU regulations and 'Council Directive 2009/158/EC of 30 November 2009 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs'. PHP also includes testing for *Mycoplasma* and *Salmonella arizonae*. Last year, 13301 poultry species were tested through this programme (Table 1).

H5 and H7 serology testing of poultry under the EU Poultry Surveillance Scheme. Ireland uses the representative sampling method described in the Commission Implementing Decision 2010/367/EU of 25 June 2010 on

Figure 1: Backyard chickens. Photo: Cosme Sánchez-Miguel

the implementation by Member States of surveillance programmes for avian influenza in poultry and wild birds. In 2018, 10263 samples were tested for H5 and H7 HAI. Up to 2018, results have been reported to the European Commission; from 2019 onwards, results will be submitted to EFSA. Categories sampled for the EU Poultry Surveillance Scheme include:

- Broilers - Free Range
- Chicken Breeders
- Layers - Free Range
- Layers - Non-Free Range
- Fattening Turkeys
- Turkey Breeders
- Fattening Ducks
- Fattening Geese

Passive surveillance.

PCR testing of wild birds. Wild bird surveillance for avian influenza in Ireland is risk-based. It is implemented as a passive surveillance scheme; dead, moribund or sick birds are reported to DAFM by members of the public or the National Parks and Wildlife Service (NPWS) by ringing the Avian Influenza Hotline (076 1064403) or the after-hours number (1850 200456). Birds are collected by trained personnel and submitted to Regional Veterinary Laboratories (RVL) for sampling. Samples

are then submitted to the Central Veterinary Research Laboratory (CVRL) where Avian Influenza testing is carried out. A list of species of wild birds to be targeted for surveillance for avian influenza is provided by the Commission Implementing Decision 2010/367/EU in accordance with the scientific opinion provided by EFSA. The list of wild birds to be targeted for AI surveillance (H5 HPAI) is amended according to the demographics of each country.

Until 2018, results of wild bird surveillance were submitted to the European Commission; from 2019 onwards, results will be submitted to EFSA. In 2018, 148 wild birds were tested. Of these, two white-tailed sea-eagles and one common buzzard were Avian influenza positive (H5N6), one mute swan was AI positive but not H5, H7, N1 or N8 (Table 1).

PCR testing of poultry. Avian influenza is a notifiable disease in Ireland, meaning that anyone who suspects that an animal may have the disease is legally obliged to notify DAFM. Poultry samples and carcasses are submitted routinely to RVLs and CVRL by private veterinary practitioners (PVPs) and backyard flocks owners for PCR testing for the purposes of diagnosis, screening and exports/imports. Farmers are encouraged to report suspect avian influenza cases to their local Regional Veterinary Office and to make use of their local Regional Veterinary Laboratory to aid with diagnosis of disease conditions. All data on Avian Influenza surveillance must be provided to the European Reference Laboratory (EURL) annually. Last year, 696 poultry birds were submitted and tested for AI PCR.

Table 1: Avian influenza surveillance testing during 2018 in Ireland

Type	No. Animals	No. Positive
Poultry Health Programme (AGID test)	13301	0
H5 and H7-EU Surveillance (HI test)	10263	0
Poultry - PCR	696	0
Wild birds - PCR	148	4*

* Three H5N6 and one non-HPAI

Avian Mycoplasma Surveillance

Active surveillance. The Poultry Health Programme operated by DAFM includes surveillance for poultry mycoplasmosis. *Mycoplasma spp.* in poultry, whilst of no public health concern, can present significant problems, both commercially and, potentially, for bird welfare.

Therefore, poultry are screened for *Mycoplasma gallisepticum* and/or *Mycoplasma meleagridis*.

Mycoplasma gallisepticum **(MG)** This mycoplasma is associated with chronic respiratory disease. Typically, it is slow in onset and can result in significant commercial losses. This mycoplasma can infect chickens, turkeys and game birds. Ducks and geese can also become infected, particularly when associated with infected chickens.

Mycoplasma meleagridis **(MM)** With this mycoplasma vertical transmission through eggs can be a significant factor. It is a disease of breeding turkeys with clinical signs possible in progeny chicks. Respiratory symptoms are the main cause of economic loss.

DAFM Poultry Health Programme seeks to provide a surveillance platform for MG and MM. As part of this programme, breeding flocks are routinely tested for serological evidence of MG (turkeys and chickens) or MM (turkeys only). The plan for each poultry subgroup varies but, typically, flocks are subject to serological testing at pre-movement (from rearing), point of lay and during production (typically every 12 *weeks*).

Frequency of sampling is set out in the *Council Directive 2009/158/EC of 30 November 2009 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs*, and the *EU commission Decision 2011/214/EU*. Sample size is based on a representative sampling strategy: 60 birds per house in houses of 1000 birds or more, with design prevalence of 5 *per cent*.

In 2018, 30620 and 1730 serum samples were screened for *M. gallisepticum* and *M. meleagridis*, respectively, at CVRL as part of DAFM official sampling (Table 2).

Passive surveillance. In addition to *M. meleagridids* and *M. gallisepticum*, *M. synoviae* is also tested as passive surveillance. In Ireland, these three serotypes are notifiable diseases, meaning that anyone who suspects an animal may have this disease is legally obliged to notify DAFM.

Beyond disease reporting, DAFM operates a network of RVLs, strategically located around the country. Farmers and PVPs are encouraged to report suspects to their local Regional Veterinary Office and to make use of their local RVL to aid with diagnosis of disease conditions.

Table 2: Official sampling for Poultry Health Programme and EU AI Surveillance during 2018 in Ireland

Submission type	Test	No. Tests	No. Positive
	M. gallisepticum SPAT	**30620**	0
Poultry Health Program	Avian Influenza AGID	**13301**	0
	M. meleagridis SPAT	**1730**	0
	Salmonella arizonae H SAT	**1400**	0
EU-H5 H7 HI-Surveillance	Avian Influenza H5	**10263**	0
	Avian Influenza H7	**10263**	0

Note:
AI Surveillance during 2018 in Ireland

Avian Salmonella surveillance

As part of the national Poultry Health Programme, serological testing for screening of *Salmonella enterica* subsp. *arizonae* is carried out in turkey flocks (Table 2). Last year, 1400 serum samples were screened for *S. arizonae*.

Table 3: Number of *Salmonella* culture tests from on-farm samples during 2018 in Ireland

Avian Production Type	No. Animals	No. Positive
Broiler Breeder	826	**0**
Layer	413	**0**
Broiler	127	**2***
Turkey Fattener	58	**13****
Broiler Grandparent	42	**0**
Turkey Breeder	12	**0**
Layer Breeder	4	**0**

[a] S. Braenderup and S. Kentuky;

[b] 11 cases of S. Derby in two sites and 2 of S. Senftenberg

In parallel, every year, DAFM carries out the EU-*Salmonella* Surveillance by collecting samples on-farm and confirming detected serotypes by culture. Programme is as follows:

- In at least one flock of broilers on 10 *per cent* of commercial broiler premises with at least 5000 birds.

- Three times a year for all broiler breeder premises with at least 250 birds

- In at least one flock per year per layer holding comprising at least 1000 birds

- Once a year in one flock on 10 *per cent* of holdings with at least 500 fattening turkeys

- Once a year in all flocks on 10 *per cent* of holdings with at least 250 adult breeding turkeys between 30 and 45 weeks of age and all holdings with elite, great grandparents and grand-parent breeding turkeys.

In 2018, 1482 samples from farms were analysed; of these, 2 cases were detected in broiler breeder farms and 13 in turkey fattener flocks by culture (Table 3).

Newcastle Disease

Newcastle Disease is a notifiable disease that affects poultry, it is caused by virulent strains of *Avian Avulavirus 1* -AAvV-1- (prior called *Avian Paramixovirus type 1* -APMV1-). A similar variant, *Pigeon AvV-1* (PPMV1) infects pigeons and other wild birds. AAvV-1 infections present a wide range of clinical signs depending of strain virulence; from lethargy and mild respiratory signs, to egg drop production, neurological signs and sudden death. Every year, samples from suspected cases and carcasses from poultry are submitted to CVRL and RVLs for ND testing. In addition, wild bird carcasses are screened by PMV1 as a means of passive surveillance. In 2018, a total of 74 wild birds and 209 poultry were tested; of these, 7 pigeons were positive (high virulent strain) as well as 2 sparrow hawks and 1 Black-Headed Gull; they were confirmed by PPMV1 PCR.

DISEASE DIAGNOSTICS

Beyond the active and passive surveillance of important notifiable diseases, DAFM carries out testing of other notifiable and non-notifiable diseases that have significant economic impact. Samples from suspect and healthy animals, the latter for monitoring purposes, are submitted directly to CVRL (Table 4) and carcasses of animals are submitted to RVLs (Tables 5 and 6, and Figure 2).

Last year, 3 birds were confirmed positive for *C. psittaci*, 16 for Infectious Bronchitis, 126 for *M. synoviae* and 4 for *M. gallisepticum* by PCR (Table 4). These diseases are notifiable and, when suspected, tissue samples or carcasses should be submitted to RVLs or CVRL. In addition, some agents such as *C. psittaci* have zoonotic potential (see textbox).

Table 4: PCR testing of submitted samples during 2018 in Ireland

Pathogen	No. Tests	No. Positive
Avian pneumovirus	12	0
Chlamidia psittaci*	41	3
Infectious Bronchitis*	599	16
Infectious largyngotracheitis*	21	0
Mycoplasma synoviae*	621	128
Mycoplasma gallisepticum*	661	4

* Notifiable diseases

C. psittaci

Psittacosis in humans is caused by *Chlamydia psittaci*, an obligate intracellular Gram-negative bacterium whose natural reservoirs are bird pets, wild birds and poultry species (pigeons, turkeys, ducks). Psittacosis has worldwide distribution and infection in humans occurs by inhalation of aerosols or direct contact with respiratory secretions or faeces of infected birds.

In humans, symptoms include flu-like symptoms such as fever, headache, and cough. Complications have been described such as pneumonia, endocarditis, hepatitis, arthritis, keratoconjunctivits and encephalitis. In humans disease can be controlled by using of antibiotics. Incubation period varies from 1–30 *days*; however, spread among humans is rare. In suspect human cases, local GPs and HSE should be contacted for advice.

In birds, symptoms can vary from unapparent to sudden death. It typically causes respiratory and/or liver problems or gastrointestinal symptoms, depending on species, age of birds and virulence of bacterial strain. In some cases, disease is carried by birds in a latent state, while still shedding bacteria, and then re-activated under immunosuppression or stressful conditions. When handling infected birds, high biosafety measures should be used such as gloves, protective eyewear and fitted respiratory mask.
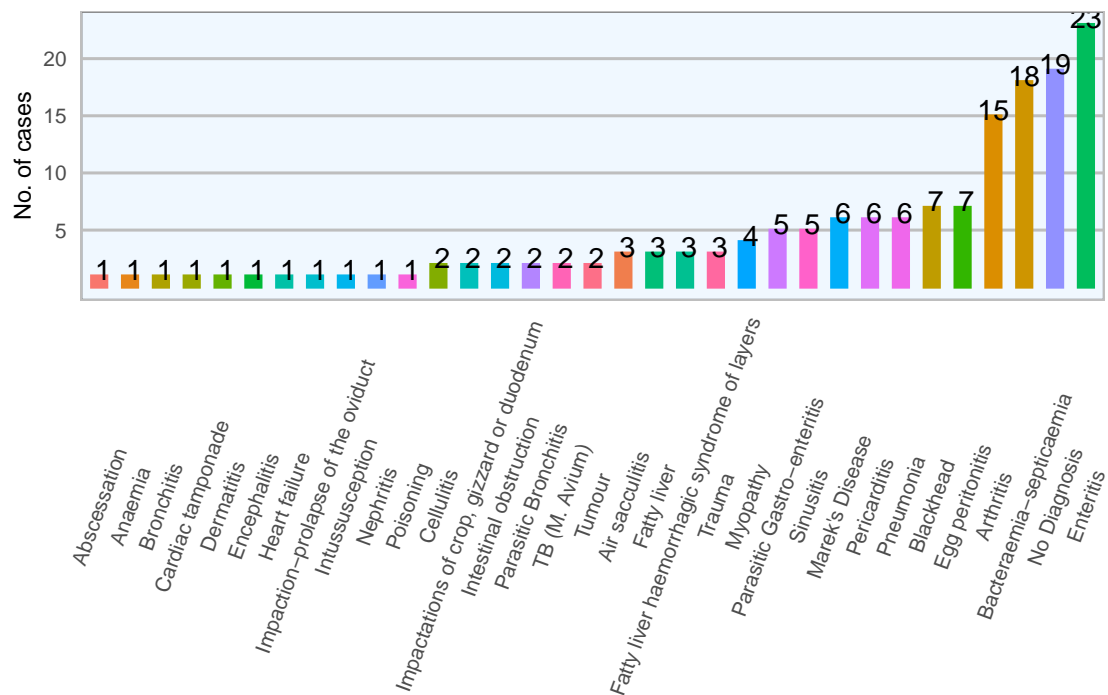
In 2018, the most common diagnosis in poultry carcasses was enteritis (23 cases) followed by bacteraemia-septicemia, arthritis and blackhead (Table 5). In wild birds, however, poisoning and PPMV1 infection were the most common diagnosis (Table 6). In a number of carcasses the cause of death was not determined.

Table 5: Diagnoses in poultry carcasses during 2018

Diagnosis	Count
Enteritis	23
No Diagnosis	19
Bacteraemia-septicaemia	18
Arthritis	15
Blackhead	7
Egg peritonitis	7
Pneumonia	6
Pericarditis	6
Marek's Disease	6
Parasitic Gastro-enteritis	5
Sinusitis	5
Myopathy	4
Trauma	3
Air sacculitis	3
Fatty liver	3
Fatty liver haemorrhagic syndrome of layers	3
TB (M. Avium)	2
Cellulitis	2
Intestinal obstruction	2
Parasitic Bronchitis	2
Tumour	2
Impactations of crop, gizzard or duodenum	2
Poisoning	1
Cardiac tamponade	1
Abscessation	1
Anaemia	1
Bronchitis	1
Dermatitis	1
Encephalitis	1
Heart failure	1
Impaction-prolapse of the oviduct	1
Intussusception	1
Nephritis	1

Case reports in poultry

Tetratrichomonas gallinarum. *Tetratrichomonas gallinarum* infection was suspected in liver samples submitted from a 10 *week* old red legged partridge reared for game. Losses were low at about 10 birds/10000 birds. Caecal casts were noted on *post-mortem* examination. Multifocal lymphohistiocytic hepatitis was observed. Presence of trichomonads, which include *T gallinarum*, was confirmed using in situ hybridization. Liebhart *et al.* (2014) reported *T. gallinarum* infection in a flock of 2500 red-legged partridges in United Kingdom and characterised by sudden death of 15 birds within 2 *days*; circulation of a virulent strain of *T. gallinarum* in reared red-legged partridges was demonstrated. It is thought that infection is by contaminated food and could be associated with unsanitary feeding places (Amin *et al.*, 2014).

Figure 2: Diagnoses recorded in poultry *post-mortem* examinations

Table 6: Diagnoses in wild bird carcasses during 2018

Diagnosis	Count
No Diagnosis	39
Poisoning	18
PPMV-1	10
Trauma	6
Parasitic gastro-enteritis	3
Avian Influenza	3
Amyloidosis	2
Malnutrition	2
Peritonitis	2
Bacteraemia-septicaemia	2
TB (M. Avium)	2
Cardiac tamponade	1
Enteritis	1
Oesophagitis	1
Arthritis	1
Pneumonia	1
Psittacosis	1
Fungal infection	1
Megabacteriosis	1

Gallibacterium anatis. *G anatis* is a normal inhabitant of the upper respiratory tract and lower reproductive tract of chickens but is also considered to be a cause of salpingitis and peritonitis in laying hens (Deplazes *et al.*, 2016). Salpingitis due to *Gallibacterium anatis* was diagnosed in an eight *month* old backyard hen, from a flock of 50 where fifteen hens had died over a 2 month period. This hen also had hepatic amyloidosis (most likely secondary to chronic systemic inflammation), colisepticaemia and granulomatous typhlitis due to presence of invasive trichomonad protozoa. Ascending infection from cloaca is suspected.

Enterococcus caecorum. Over the past 15 *years*, pathogenic strains of *E. caecorum* have become a significant cause of morbidity and mortality in broiler breeders. Repeated outbreaks occur. An environmental reservoir for pathogenic E. caecorum has yet to be identified (Jung *et al.*, 2018). An outbreak of *Enterococcus caecorum* infection in 21 *day* old broilers was diagnosed. Birds presented with splayed legs and lameness, resulting in excess culling. They responded to antibiotics but when antibiotic administration ceased the problem returned. In some birds, the neck of the femur fractured easily on disarticulation. *E. caecorum* was cultured from pericardium, bone and joint. Affected birds had pyogranulomatous epicarditis, arthritis, tenosynovitis, osteomyelitis and chondritis.

Chronic cholera. Fowl cholera is more likely to occur in birds that are stressed by e.g. parasitism, poor hygiene, malnutrition and other diseases. Chronic cholera was diagnosed in 62 *week* old free range layers which were wasting and experiencing ongoing mortality. One bird, which presented with opisthotonus and twitching, had pyogranulomatous and fibrinous osteomyelitis at the base of the skull with adjacent cellulitis. Another bird had severe peritonitis and oophoritis with inspissated pus in ovaries. *Pasteurella multocida* was cultured from ovary, oviduct, liver and abdomen. A predisposing cause was not found in this outbreak.

Ornithobacterium rhinotracheale. *Ornithobacterium rhinotracheale* (ORT) is of worldwide distribution in commercial poultry, in which it is associated with respiratory diseases, and is also found in wild birds. Airsacculitis and pneumonia are the most common features of infection with ORT. Infection can be transmitted horizontally by aerosol and vertically through eggs, which probably accounts for its rapid and worldwide spread (van Empel and Hafez, 1999). ORT was isolated from a 36 *day* old organic broiler from a group which had Marek's disease. This bird had cheese like material in its air sac. Other infections in the birds examined, which were likely due to virus induced immunosuppression, included coccidiosis, cryptosporidiosis and *Gallibacterium anatis* associated pneumonia.

References

Amin A, Bilic I, Liebhart D, Hess M (2014). "Trichomonads in birds – a review." *Parasitology*, **141**(6), 733–747. doi: 10.1017/S0031182013002096. URL https://www.cambridge.org/core/journals/parasitology/article/trichomonads-in-birds-a-review/AA8C93C49511537CD1AF9EA82DD34E6F.

Deplazes P, Eckert J, Mathis A, von Samson-Himmelstjern G, Zahner H (2016). *Parasitology in veterinary medicine.* Wageningen Academic Publishers.

Jung A, Chen LR, Suyemoto MM, Barnes HJ, Borst LB (2018). "A Review of Enterococcus cecorum Infection in Poultry." *Avian Diseases*, **62**(3), 261–271. doi:10.1637/11825-030618-Review.1. https://doi.org/10.1637/11825-030618-Review.1, URL https://doi.org/10.1637/11825-030618-Review.1.

Liebhart D, Neale S, Garcia-Rueda C, Wood AM, Bilic I, Wernsdorf P, Jaskulska B, Hess M (2014). "A single strain of Tetratrichomonas gallinarum causes fatal typhlohepatitis in red-legged partridges (Alectoris rufa) to be distinguished from histomonosis." *Avian Pathology*, **43**(5), 473–480. doi:10.1080/03079457.2014.959435. PMID: 25175532, https://doi.org/10.1080/03079457.2014.959435, URL https://doi.org/10.1080/03079457.2014.959435.

van Empel PCM, Hafez HM (1999). "Ornithobacterium rhinotracheale: A review." *Avian Pathology*, **28**(3), 217–227. doi:10.1080/03079459994704. PMID: 26915377, https://doi.org/10.1080/03079459994704, URL https://doi.org/10.1080/03079459994704.

TB and Deer in Ireland

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Tuberculosis, a chronic progressive disease which results in considerable production losses in cattle as well as in other species, is caused by the bacterium *Mycobacterium bovis*. This agent may be transmitted in a number of ways by infectious animals.
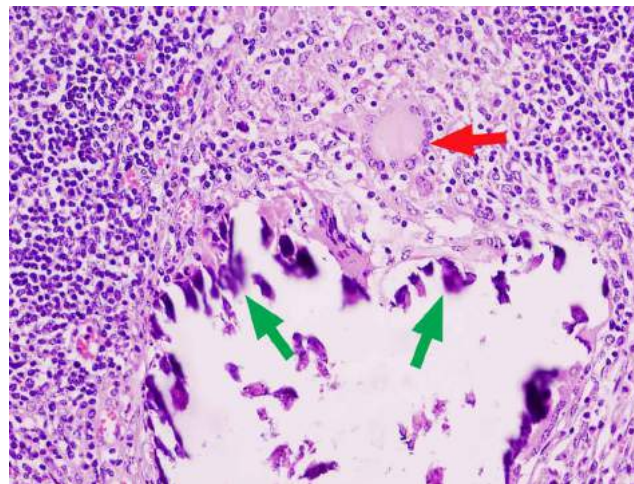
Wildlife reservoirs have been implicated as a source of infection for grazing cattle. Infected badgers are considered maintenance hosts and are directly implicated in the transmission of *Mycobacterium bovis* to cattle in Ireland (Griffin *et al.*, 2005). With respect to deer, their role in acting as a maintenance host for *Mycobacterium bovis* is considerably less clear, in most areas of Ireland, there is no evidence in support of deer acting as maintenance host for *TB* (More, 2019).

In certain areas of County Wicklow, a higher prevalence of *TB* in deer has been found. Recently, DAFM, in cooperation with the NPWS and local stakeholders groups, has been carrying out research into the prevalence of *TB* in deer populations. The findings of these ongoing studies will be reported in due course.

Between 2018 and 2019 Tralee RVO led an investigation into *TB* in deer within its management region. To this end, 184 separate deer carcass specimens were sent from the south west of Ireland for post mortem examination to Cork RVL. Most deer specimens were comprised of head and pluck (composed heart and lungs), liver and kidneys; the remainder consisted of heads only.

Post mortem examination involved dissection and inspection of lymph nodes in the head (submandibular, parotid, and retropharyngeal), bronchial and mediastinal lymph nodes, lungs, liver, hepatic lymph node and kidneys when available.

Where suspect gross lesions suggestive of *TB* were found, these were sampled and submitted to the *TB* Section at the CVRL Backweston for histopathology and/or bacteriological culture testing.

Figure 1: Chronic granulonatous inflammation. Langhan's multinucleated giant cell (red arrow) and dystrophic calcification (green arrows) due to necrosis in a TB granuloma. Photo: Cosme Sánchez-Miguel.

Of all of the cervine samples submitted, one sample yielded *Mycobacterium bovis* on culture, a second sample yielded an atypical non-tuberculous *Mycobacterium spp.*; the remaining samples had negative cultures. In addition, histopathological examination of these samples did not reveal any lesion suggestive of *TB*, corroborating bacteriological findings.

In conclusion, of the 184 carcass specimens submitted to the DAFM laboratory service from Tralee RVO, *Mycobacterium bovis* infection was confirmed in one submission, a prevalence of 0.54 *per cent*.

During the period 2014–2016, Drumshanbo RVO led an epidemiological study of *TB* in wildlife, as part of its enhanced response to an outbreak of *TB* in cattle herds in north Co. Sligo. As part of the investigation, 145 badgers were culled in the vicinity of *TB* breakdown herds and were subjected to both gross post mortem examination and bacterial culture of lymph node tissue. Tuberculosis was confirmed in 26.2 *per cent* of these badgers. In addition to the badger work, 17 deer from the same study area shot under licence from the NPWS were subjected to similar testing, none were found to have *TB* (Doyle *et al.*, 2018)

This low *TB* prevalence was reflected in the findings of the largest post mortem survey (and therefore statis-

Table 1: Outline of investigations in Ireland into prevalence (%) of TB in deer since 1997, not including Wicklow studies which will be reported when available

Survey source	Time period	Deer	Type	TB	Prevalence
TB lab CVRL	1997	14842	Farmed	41	0.28
TB lab CVRL	1997	340	Wild	9	2.80
Drumshanbo RVO	2014 – 2016	17	Wild	0	0.00
Tralee RVO	2018 – 2019	184	Wild	1	0.54

tically most robust) of *TB* in deer carried out in Ireland. A 1997 publication by Quigley *et al.* (1997) describes this study where 14,842 farmed deer were slaughtered and examined post mortem in Irish abattoirs between January 1993 and September 1996. Lymph nodes in the head and pluck were visually examined and gross lesions were removed, submitted to the laboratory for histopathology and cultured. *TB* caused by *Mycobacterium bovis* was confirmed in 41 of the slaughtered deer, a 0.28 *per cent* prevalence. The same study looked at 340 wild deer culled from Glenveagh National Park Co. Donegal and found a prevalence of *Mycobacterium bovis* infection of 2.8 *per cent*.

Several DAFM coordinated studies have been carried out to estimate the prevalence of *TB* in deer in Ireland. The largest of these studies found a *TB* prevalence of 0.28 *per cent* in farmed deer. Two recent epidemiological studies, led by two separate Regional Veterinary

Offices, found *TB* incidences of a similarly low order, 0.54 *per cent* in the south west and 0 *per cent* in North Sligo. Data from Co. Donegal outlined above suggest a somewhat higher, albeit still low, *TB* prevalence in wild deer. Research from Wicklow is ongoing and will be reported when completed.

Over many years, research into *TB* in deer has focussed on disease prevalence, perhaps future research, using newer research tools, should focus on dynamics, ecological drivers and the spread of infection, both within deer populations and between deer and other mammalian species.

References

Doyle R, Clegg TA, McGrath G, Tratalos J, Barrett D, Lee A, More SJ (2018). "The bovine tuberculosis cluster in north County Sligo during 2014-16." *Irish Veterinary Journal*, **71**(1), 24. ISSN 2046-0481. doi:10.1186/s13620−018−0135−z. URL https://doi.org/10.1186/s13620-018-0135-z.

Griffin J, Williams D, Kelly G, Clegg T, O'Boyle I, Collins J, More S (2005). "The impact of badger removal on the control of tuberculosis in cattle herds in Ireland." *Preventive Veterinary Medicine*, **67**(4), 237 – 266. ISSN 0167-5877. doi:https://doi.org/10.1016/j.prevetmed.2004.10.009. URL http://www.sciencedirect.com/science/article/pii/S0167587704002090.

More SJ (2019). "Can bovine TB be eradicated from the Republic of Ireland?: Could this be achieved by 2030?" *Irish Veterinary Journal*, **72**(1), 3. ISSN 2046-0481. doi:10.1186/s13620−019−0140−x. URL https://doi.org/10.1186/s13620-019-0140-x.

Quigley FC, Costello E, Flynn, Gogarty A, McGuirk J, Murphy A, Egan J (1997). "Isolation of mycobacteria from lymph node lesions in deer." *Veterinary Record*, **1**(141), 516–518. URL https://veterinaryrecord.bmj.com/content/vetrec/141/20/516.full.pdf.

Agri-Food & Biosciences Institute (AFBI)

Disease Surveillance and Investigation Branch

2018

All-Island Animal Disease Surveillance Report

Animal Disease Surveillance at the Agri-Food & Biosciences Institute (AFBI)

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AFBI provides animal disease surveillance and diagnostic services for the Northern Irish farming industry. Passive (scanning) surveillance is carried out by collecting and analysing data from post mortem and clinical samples submitted to the Omagh and Stormont laboratory sites of the Disease Surveillance and Investigation Branch (DSIB) of AFBI. Analysis of this data assists in establishing baselines for incidence of endemic disease, facilitating recognition of new and emerging diseases and allowing rapid identification of changing trends in disease prevalence. This allows evidence based decision making by veterinary providers and at national level.

Cattle Diseases

Neonatal Calves (0–1 *months*). As in previous years, enteric infections were the most frequently diagnosed cause of death for this age group in 2018 in calves submitted to AFBI for *post mortem* (see Table 1 and Figure 1 and the breakdown on bovine neonatal enteritis in Table 9 and Figure 15).

Failure of passive transfer of maternal immunity was detected in 74 *per cent* of blood samples tested using ZST (zinc sulphate turbity test) from calves aged less than 10 *days* (Figure 16), highlighting the importance of good colostrum management. This year respiratory infections were the second most commonly diagnosed cause of death for this age group.

Calves (1–5 *months*). In 2018, respiratory infections accounted for just under half (46.6 *per cent*) of all deaths in this age group in calves submitted to AFBI for *post mortem* (Table 2 and Figure 3), highlighting the economic and welfare implications of respiratory disease (see also Table 6 and Figure 10 in the *Bovine Respiratory Disease* section below).

Bacterial causes of pneumonia were the most frequently diagnosed respiratory infections at AFBI in 2018. *Mycoplasma bovis* was the most frequently recorded pathogen and was detected in 29 *per cent* of respiratory infections, followed by *Pasteurella multocida* (14 *per cent* of respiratory infections). *Mannheimia haemolytica* was diagnosed in 7 *per cent* of respiratory infections and *Trueperella pyogenes* was isolated in 6 *per cent* of cases. Bovine respiratory syncytial virus
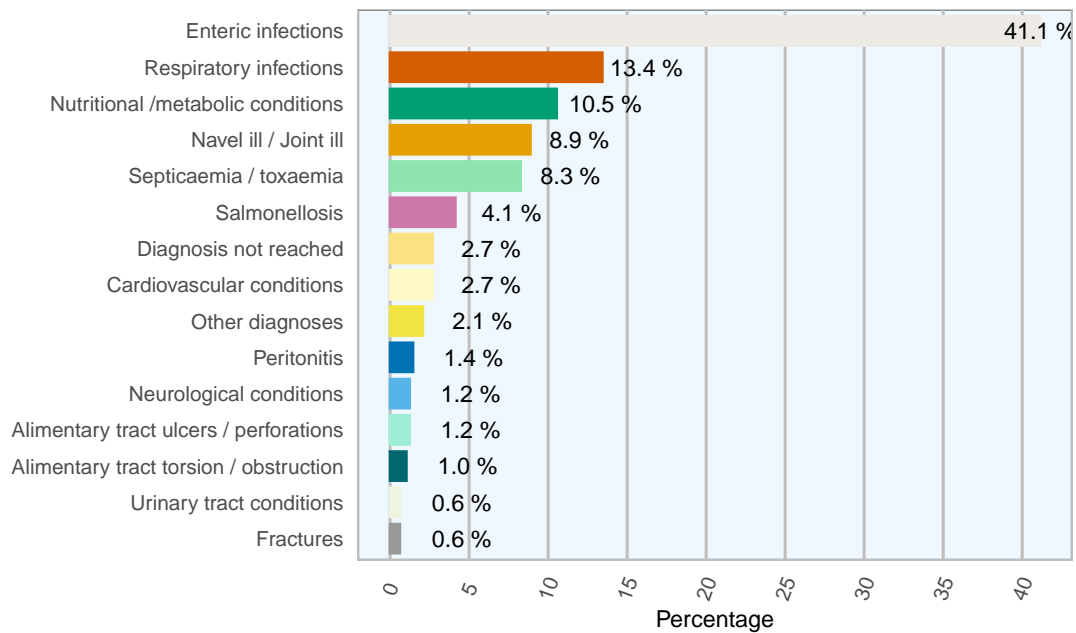
Table 1: Conditions most frequently diagnosed on *post-mortem* examinations of neonatal calves (0–1 months) by AFBI during 2018 (n= 484).

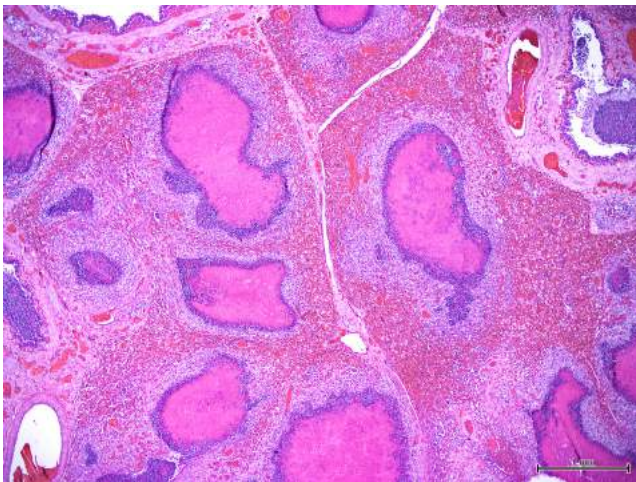| Category	No. of cases	Percentage
Enteric infections	199	41.1
Respiratory infections	65	13.4
Nutritional /metabolic conditions	51	10.5
Navel ill / Joint ill	43	8.9
Septicaemia / toxaemia	40	8.3
Salmonellosis	20	4.1
Cardiovascular conditions	13	2.7
Diagnosis not reached	13	2.7
Other diagnoses	10	2.1
Peritonitis	7	1.4
Alimentary tract ulcers / perforations	6	1.2
Neurological conditions	6	1.2
Alimentary tract torsion / obstruction	5	1.0
Fractures	3	0.6
Urinary tract conditions	3	0.6

Table 2: Conditions most frequently diagnosed on *post-mortem* examinations of calves (1-5 months) by AFBI during 2018 (n= 326).

Category	No. of cases	Percentage
Respiratory infections	152	46.6
Alimentary tract torsion / obstruction	25	7.7
Diagnosis not reached	23	7.1
Enteric infections	21	6.4
Clostridial disease	16	4.9
Other diagnoses	13	4.0
Navel ill / Joint ill	12	3.7
Nutritional / metabolic conditions	12	3.7
Septicaemia / toxaemia	11	3.4
Alimentary tract ulcers / perforations	8	2.5
Neurological conditions	8	2.5
Cardiovascular conditions	7	2.1
Poisoning	7	2.1
Peritonitis	4	1.2
Urinary tract conditions	4	1.2
BVD/Mucosal disease	3	0.9

(BRSV) was the most frequently diagnosed viral cause of infection in this age of calves and was detected in 9 *per cent* of cases and was thus the third most frequently recorded respiratory pathogen in 2018. Infec-

Figure 1: Conditions most frequently diagnosed on *post-mortem* examinations of neonatal calves (0–1 months) by AFBI during 2018 (n= 484).

Figure 2: Pneumonia with multifocal caseous necrosis due to *Mycoplasma bovis* infection in a six-week-old calf. Photo: AFBI

tious bovine rhinotracheitis virus (IBRV) was detected in 1% of respiratory infections in this age group.

The importance of suitable housing, ventilation and control of stress factors in calves of this age are issues that need to be addressed in order for this situation to improve. Vaccination programmes and antibiotic care, where appropriate, should also be discussed.

Intestinal or gastrointestinal torsions accounted for 6 *per cent* of overall diagnoses in this age group. Enteric

infections were the third most frequently diagnosed cause of death in calves from one to five months of age and included infections such as cryptosporidiosis, parasitic gastroenteritis, coccidiosis and coronaviral infections.

Clostridial disease was again important in this age group despite the availability of effective clostridial vaccines to help prevent losses. Blackleg was the most commonly recorded clostridial disease in one to five month old calves representing 56 *per cent* of the clostridial diseases recorded. Botulism accounted for 19 *per cent* of the clostridial diagnoses and malignant oedema made up 12 *per cent* of clostridial disease recorded. Over 70 *per cent* of poisoning diagnoses in this age group were due to lead intoxication.

Calves (6–12 *months*). In 2018, with an incidence of 46.2 *per cent* of cases, respiratory infections were also the most commonly diagnosed cause of death in this age group (Table 3 and Figure 5), this is a slight decrease when compared to 2017, 49.1 *per cent*.

Bacterial infections were the most frequent cause of respiratory tract infections. Of the bacterial infections, *Mycoplasma bovis* was most often detected (15 cases representing 20 *per cent* of respiratory tract infections) followed by *Pasteurella multocida* (8 cases or 11*per cent* of respiratory infections), *Mannheimia haemolyt-*

Figure 3: Conditions most frequently diagnosed on *post-mortem* examinations of calves (1-5 months) by AFBI during 2018 (n= 326).

Figure 4: Cerebrocortical necrosis (CCN) in a five-month-old heifer. Photo: Sean Fee

Table 3: Conditions most frequently diagnosed on *post-mortem* examinations of calves (6–12 months) by AFBI during 2018 (n= 160).

Category	No. of cases	Percentage
Respiratory infections	74	46.2
Clostridial disease	28	17.5
Diagnosis not reached	16	10.0
Enteric infections	9	5.6
Other diagnoses	7	4.4
Cardiovascular conditions	4	2.5
Liver disease	4	2.5
Neurological conditions	4	2.5
Poisoning	4	2.5
Gastrointestinal ulceration or foreign body	2	1.2
Nutritional / metabolic conditions	2	1.2
Skeletal conditions	2	1.2
Urinary tract conditions	2	1.2
Gastrointestinal torsion	1	0.6
Peritonitis	1	0.6

ica (8 cases or 11*per cent* of respiratory infections)*, Haemophilus somni* (4 cases or 5*per cent* of respiratory infections) and *Arcanobacterium pyogenes* (2 cases) . Four cases of bovine respiratory syncytial virus and one case of IBR were recorded. Waning passive immunity derived from colostrum feeding, autumn housing, weaning, mixing and transport as well as husbandry activities such as dehorning and castration increase stress and help predispose to outbreaks of viral and bacterial respiratory disease in this age group of calves.

Despite availability of cheap and effective vaccines, clostridial diseases were identified as the second largest cause of mortality in this age group at 17.5 *per cent*.

Blackleg occurred most commonly in this age range and was diagnosed in 21 cases, while 5 cases of botulism and two cases of black disease were diagnosed. For the first time since an all-island Animal Disease Surveillance Report was first produced by AFBI and DAFM in 2010, 2018 was the first year in which no cases of BVD or mucosal disease were detected in weanling cattle in the North of Ireland demonstrating the
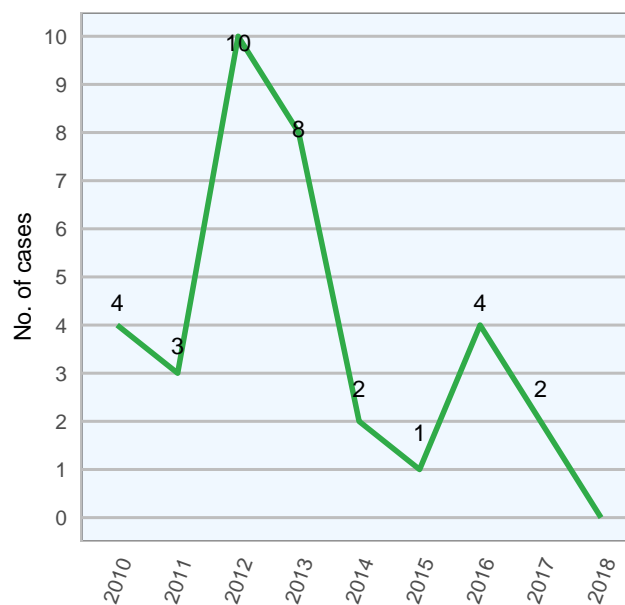
Figure 5: Conditions most frequently diagnosed on *post-mortem* examinations of calves (6-12 months) by AFBI during 2018 (n= 160).

progress being made by the BVD eradication scheme (Figure 6).

Table 4: Conditions most frequently diagnosed on *post-mortem* examinations of adults (older than 12 months) by AFBI during 2018 (n= 397).

Category	No. of cases	Percentage
Respiratory infections	89	**22.4**
Clostridial disease	51	**12.8**
Diagnosis not reached	45	**11.3**
Cardiac / circulatory system conditions	44	**11.1**
Other diagnoses	30	**7.6**
Reproductive tract infections / Mastitis	30	**7.6**
Liver disease	19	**4.8**
Peritonitis	19	**4.8**
Nutritional /metabolic conditions	18	**4.5**
Enteric infections	12	**3.0**
GIT ulceration/perforation / foreign body	10	**2.5**
Intestinal or gastric torsion / obstruction	10	**2.5**
Poisoning	7	**1.8**
Urinary tract conditions	7	**1.8**
Neurological conditions	6	**1.5**

Figure 6: The number of cases of mucosal disease in weanlings aged 6 to 12 per year diagnosed on *post-mortem* by AFBI

Adults (older than 12 *months*). As in previous years, respiratory infections were identified as the most common cause of death in adult cattle submitted to AFBI for *post mortem* (Table 4 and Figure 8), accounting to 22.4 *per cent* of the cases in this group. Clostridial dis-

Figure 7: Pale focus typical of black disease in a bovine liver. Photo: AFBI

ease accounted for 12.8 *per cent* of adult cattle deaths in 2018, representing an increase from the 8.4 *per cent* of cases in 2017 (Figure 7). Cardiac / circulatory system conditions such as endocarditis, pericarditis and caudal vena cava thrombosis were also common causes of mortality in adult cows in 2018; likely due to being notoriously difficult to identify and treat ante-mortem and their guarded to poor prognosis (Buczinski *et al.*, 2010).

Bovine Respiratory Disease (BRD)

Bovine respiratory disease was the most commonly identified cause of death in all age groups of bovines, other than neonatal calves, submitted to AFBI in 2018 and was the second most commonly identified cause of death in neonatal calves. There were multiple aetiologies identified (Table 5). The economic cost of wastage and reduced productivity due to cattle respiratory infections are considerable.

Table 5: Most common diagnostic groups on *post-mortem* examinations of bovine respiratory disease by AFBI during 2018 (n= 236).

Category	No. of cases	Percentage
Bacterial	184	78.0
Parasitic	31	13.1
Viral	21	8.9

As in 2017, in 2018 *Mycoplasma bovis* was the pathogen identified with the greatest frequency in cattle diagnosed with respiratory disease, increasing from 23.2 *per cent* to 30.9 *per cent*. At AFBI, *M. bovis* is detected by PCR tests, positive results need to be inter-preted in conjunction with gross and histopathological findings.

Mycoplasma bovis adhere closely to host cells and facilitate damage to cells by soluble factors such as hydrogen peroxide, surface protein variation allows the organism to evade developing immune response by rapidly adapting to internal host environment. *M. bovis* can cause severe pneumonia in the absence of other organisms or can add to disease where the causative pathogens are *Pasteurella spp.* or *Mannheimia spp.* (Quinn *et al.*, 2015). Good management practices and antimicrobial therapy are the most important methods of treatment and control. However, there may be a poor response if disease presentation is chronic.

M. bovis was followed in percentage by *P. haemolytica*, *P. multocida*, *D. viviparus* (lungworm), *T. pyogenes*, BRSV, *H. somnus*, IBR and BVD (Table 6 and Figure 10).
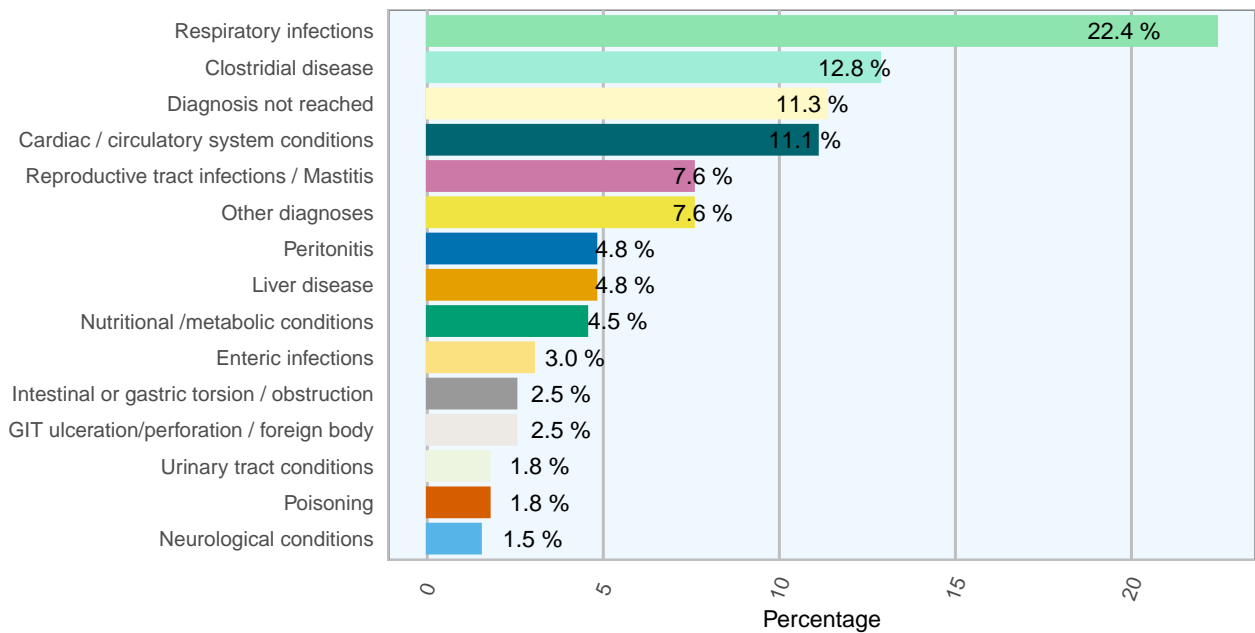
Table 6: Relative frequency of diagnoses in bovine respiratory disease recorded by AFBI during 2018 (n= 236).

Category	No. of cases	Percentage
Mycoplasma bovis	73	30.9
Pasteurella haemolytica	43	18.2
Pasteurella multocida	40	16.9
Dictyocaulus viviparus	31	13.1
Trueperella pyogenes	21	8.9
Bovine Respiratory synthical virus BRSV	16	6.8
Histophilus somnus	7	3.0
Infectious Bovine Rhinotracheitis (IBR)	4	1.7
Bovine Viral Diarrhoea (BVD)	1	0.4

Lungworm. Parasitic bronchitis due to lungworm *Dictyocaulus viviparus* (Figure 9) was seen in 13.1 *per cent* of bovine respiratory disease cases, with a seasonal peak towards November (Figure 11). In areas with a mild climate, high levels of rainfall and abundance of permanent grassland, outbreaks of this disease can occur at any stage between June and November, most commonly from July until September.

Generally, only calves in their first grazing year are clinically affected, as older animals in endemic areas generally have strong acquired immunity. Infection may persist in calves through overwintered larvae on pasture (from autumn until late spring) and carrier animals with arrested larvae persisting in the bronchi until the next grazing season. Although dairy calves are more typically affected than beef calves, autumn-born sucklers have the same level of susceptibility once they are turned out in early summer.

Adult cattle can be infected with parasitic bronchitis

Figure 8: Conditions most frequently diagnosed on *post-mortem* examinations of adults (older than 12 months) by AFBI during 2018 (n= 397).

Figure 9: Trachea containing large numbers of lungworm (*Dictyocaulus viviparus*). Photo: AFBI

if they have not had sufficient exposure in previous years to develop an adequate immune response prior to being grazed on pastures with a heavy burden of parasites.

Control can be achieved through a lungworm vaccination programme given orally to calves over 8 weeks of age and by the use of prophylactic early-season anthelmintic treatments or rumen boluses; though the latter should be considered with care so as not to deny treated animals exposure to natural challenge, which may result in susceptibility later in life.

Bovine Abortion

Bovine abortion is a significant cause of livestock wastage and results in considerable costs to the cattle industry. As a general guideline, once level of abortions exceeds 3 to 5 *per cent* or if there are several abortions clustered together, an investigation should be carried out. Specimens from 367 (Table 7 and Figure 12) bovine abortions and stillbirths were examined during 2018 (427 were examined in 2017).

Significant pathogens were detected in 153 of these, 41.7 *per cent*. This diagnostic rate is comparable to other years and reflects the multiple aetiologies of bovine abortion and the fact that not all abortions are due to an infectious cause.

Table 7: Most frequently diagnosed causes of cattle abortion diagnosed at AFBI in 2018 (n= 367).

Category	No. of cases	Percentage
No infectious agent identified	174	**47.4**
Other	40	**10.9**
Trueperella pyogenes	36	**9.8**
Salmonella spp	33	**9.0**
Bacillus licheniformis	24	**6.5**
Neospora caninum	22	**6.0**
Bovine Viral Diarrhoea	13	**3.5**
E.coli	13	**3.5**
Leptospirosis	12	**3.3**

Figure 10: Relative frequency of diagnoses in bovine respiratory disease recorded by AFBI during 2018 (n= 236).
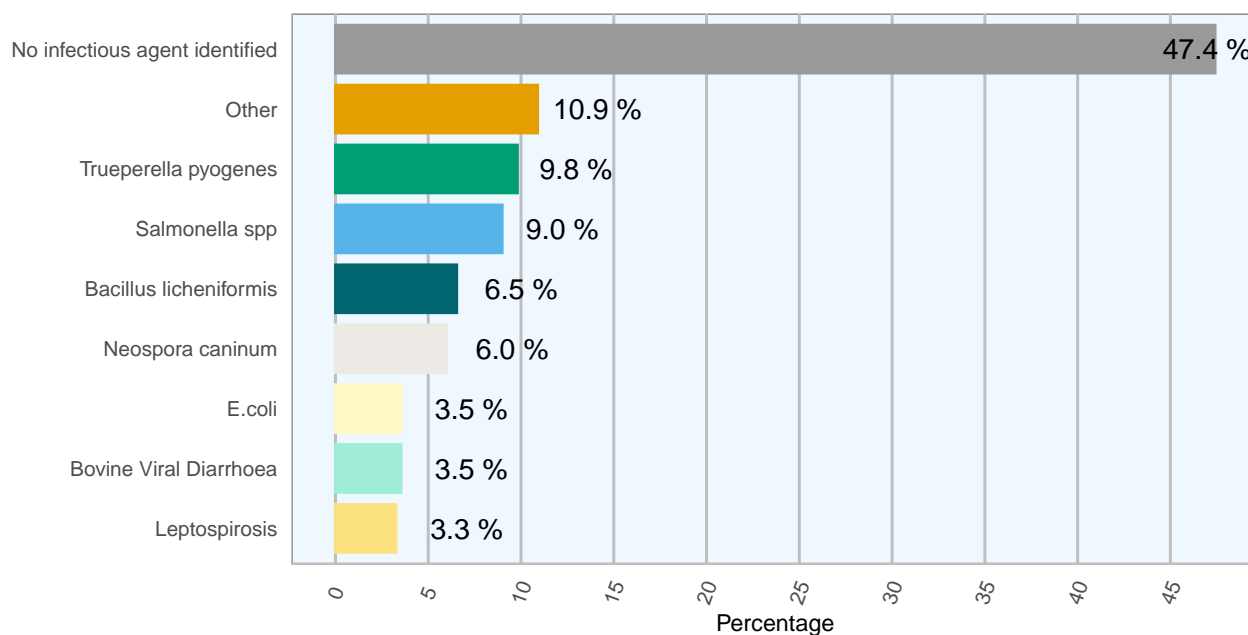

Figure 11: Annual distribution of deaths in cattle due to lungworm recorded by AFBI during 2018 (n= 39).

As in previous years, *Trueperella pyogenes*, *Salmonella Dublin* and *Bacillus licheniformis* were the most commonly diagnosed pathogens. *Neospora caninum* was the fourth most commonly diagnosed pathogen followed by BVDV and Leptospirosis. *S. Dublin* presented a characteristic annual distribution with an increase at the end of the year with a peak in November (Figure 13).

T. pyogenes is considered a sporadic cause of abortion through haematogenous spread and placentitis. It is suspected that the organism penetrates into the bloodstream from mucosal surfaces and, when the animal is pregant, can result in placental localisation and abortion; this occurs primarily in the last trimester but, potentially, at any stage of gestation. Illness in dams usually follows this kind of abortion and may prove fatal due to mastitis, arthritis or suppurative endometritis.

Bacillus licheniformis also results in necrosuppurative placentitis but, unlike *T. pyogenes*, does not cause any signs of systemic illness in the cow. *B. licheniformis* is ubiquitous, although silage, run-off water, foodstuffs and bedding that have become contaminated with silage effluent and wet spoiled hay are the most likely sources of infection through suspected haematogenous entry from the gastrointestinal tract.
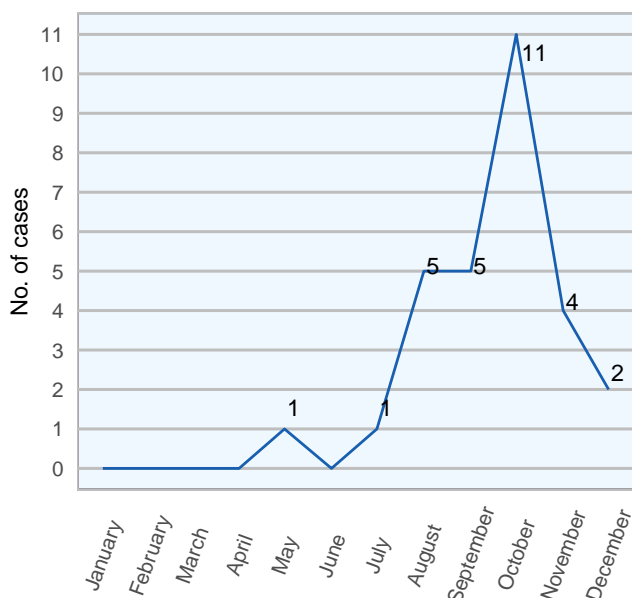
Figure 12: Most frequently diagnosed causes of cattle abortion diagnosed at AFBI in 2018 (n= 367).

Bovine Mastitis

Figure 13: *Salmonella Dublin* abortions in cattle recorded by AFBI during 2018 (n= 29).

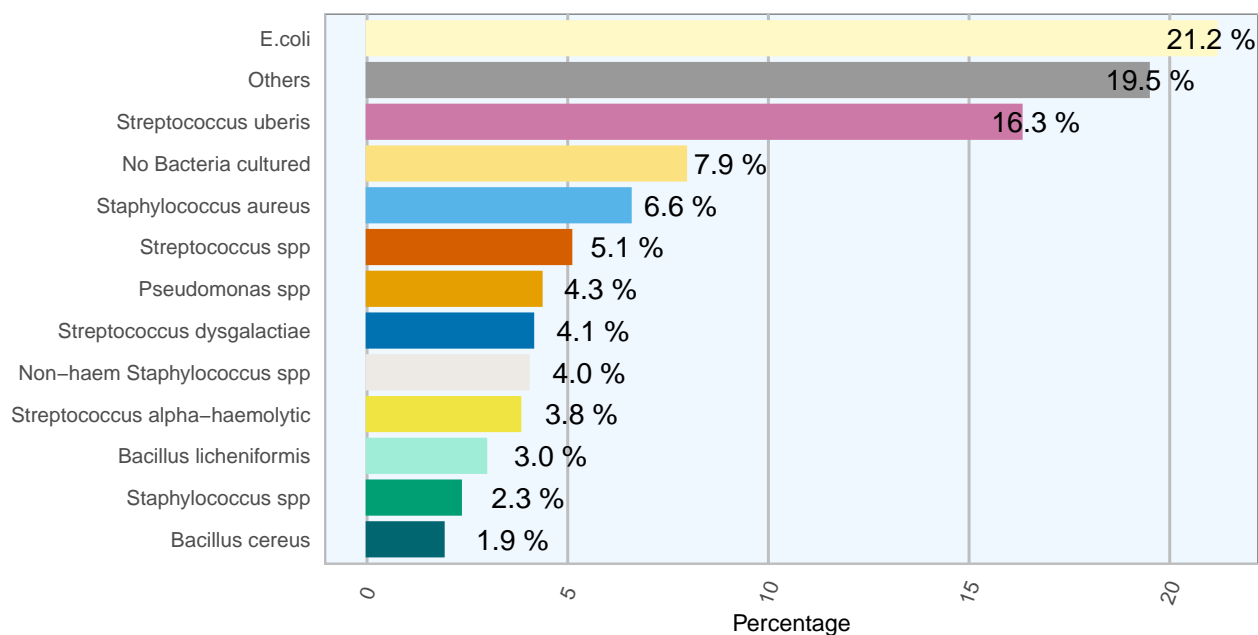**Table 8:** Most frequently isolated bacteria from milk samples submitted to AFBI in 2018 (n= 945).

Category	No. of cases	Percentage
E.coli	200	**21.2**
Others	184	**19.5**
Streptococcus uberis	154	**16.3**
No Bacteria cultured	75	**7.9**
Staphylococcus aureus	62	**6.6**
Streptococcus spp	48	**5.1**
Pseudomonas spp	41	**4.3**
Streptococcus dysgalactiae	39	**4.1**
Non-haem Staphylococcus spp	38	**4.0**
Streptococcus alpha-haemolytic	36	**3.8**
Bacillus licheniformis	28	**3.0**
Staphylococcus spp	22	**2.3**
Bacillus cereus	18	**1.9**

A total of 945 bacterial isolates were cultured from milk samples submitted from acute and chronic mastitis cases (Table 8 and Figure 14). Significance of the organism depends on cell count, organism isolated and whether or not this organism was isolated in pure culture. Isolation of 3 or more species suggests contamination during sampling. Result interpretation should, therefore, be undertaken with care.

E.coli was the most frequently isolated organism accounting for 21.2 *per cent* of isolates cultured, com-

Figure 14: Relative frequency of most commonly identified infectious causes of mastitis in milk samples submitted to AFBI in 2018 (n= 945).

pared with 22.8 *per cent* in 2017. *E.coli* is the most prevalent environmental cause of mastitis and produces opportunistic infections in animals with reduced immune status or animals with heavy exposure to pathogenic strains of the bacteria. Infection primarily occurs when the teat canal is open. Risk factors include poor hygiene, suboptimal milking machine function, teat-end damage and lactation.

Another frequently identified environmental organism, *Streptococcus uberis*, is a naturally resident of skin, tonsillar tissue and vaginal mucosa and was identified in 16.3 *per cent* of submitted samples, a decline on previous years. Contamination usually occurs in housed animals due to poor environmental hygiene.

Staphylococcus aureus, a *contagious* cause of mastitis that typically spreads from cow to cow via milking equipment and contaminated milker's hands, was identified in 6.6 *per cent* of samples submitted in 2018, compared to 8.5 *per cent* in 2017. Most *S. aureus* infections are subclinical, though they can vary from peracute life-threatening systemic forms to chronic infections which lead to elevated somatic cell counts, abscessation and fibrosis of collecting ducts. Chronic, low-grade and subclinical infections are a significant cause of economic losses through a drop in production and the withholding of milk during antibiotic treatment.
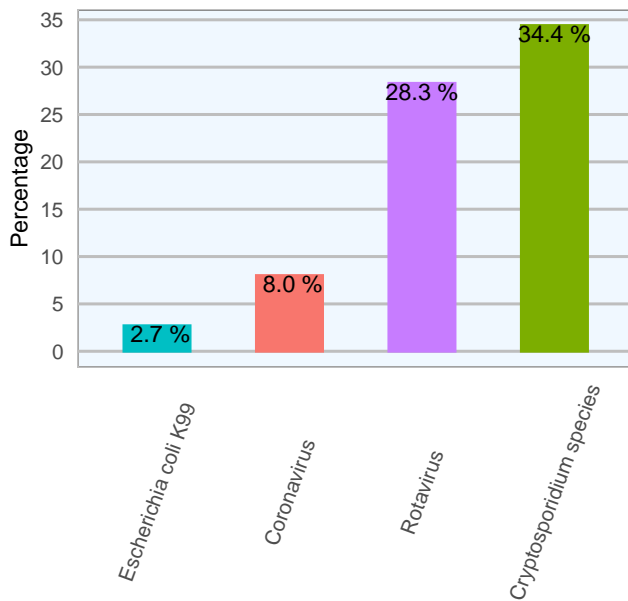
Bovine Neonatal Enteritis

Table 9: Frequency of common enteropathogenic agents identified in calf faecal samples tested by AFBI in 2018.

Organism	No. Tested	Positive	Percentage
Cryptosporidium species	822	283	**34.4**
Rotavirus	823	233	**28.3**
Coronavirus	835	67	**8.0**
Escherichia coli K99	447	12	**2.7**

Approximately 850 neonatal faecal samples were examined by AFBI in 2018 (Table 9 and Figure 15). As in previous years, *Cryptosporidium spp.* was the most frequently identified pathogen, 34.4 *per cent*, *Rotavirus* was the second, 28.3 *per cent*, and *Coronavirus* the third, 8 *per cent*. Faecal samples sent to AFBI from calves younger than two weeks of age are also checked for *E.coli* expressing the *K99* fimbrial antigen. *E.coli K99* has been associated with enterotoxigenic colibacillosis in neonates. In 2018, E.coli K99 was identified in 2.7 *per cent* of faecal samples from calves younger than 2 *weeks* of age.

Neonatal calves are most susceptible to infection during the first few days of life with watery diarrhoea being the usual first clinical sign. The low pH of the distal small intestine provides the ideal growth environment for pathogens, which colonise the gut epithelium and

Figure 15: Frequency of common enteropathogenic agents identified in calf faecal samples tested by AFBI in 2018.

Figure 16: Results of Zinc Sulphate Turbidity tests performed by AFBI in 2018 from bovine calf serum samples taken at *post-mortem* (n= 188). Adequate colostral immunity is defined as equal or greater than to 20 *units*.
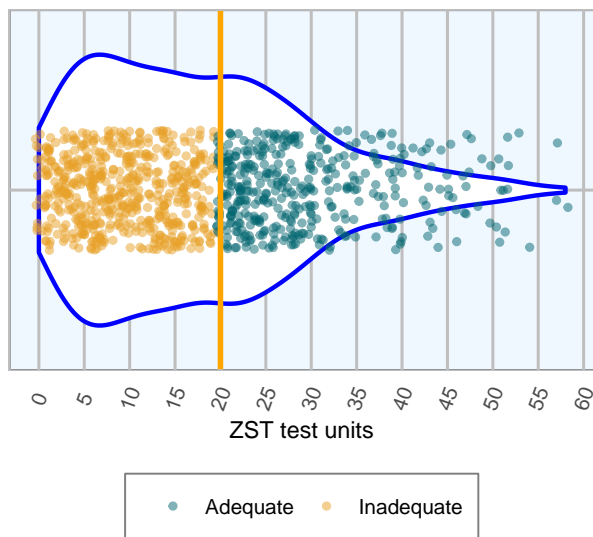
cause villous atrophy and up-regulation of chloride secretion, pulling water into the lumen of the intestine by osmosis and resulting in the classic secretory diarrhoea (Malmuthuge and Guan, 2017).

Zinc Sulphate Turbidity (ZST) Test

Table 10: Results of Zinc Sulphate Turbidity tests performed by AFBI in 2018 from bovine calf serum samples (n= 1156).

Submission	Status	Count	Mean	Percentage
Diagnostic	Adequate	413	30.1	**43**
	Inadequate	555	9.3	**57**
Post-mortem	Adequate	49	32.3	**26**
	Inadequate	139	9.9	**74**

The Zinc Sulphate Turbidity test is an indirect measurement of the passive transfer of immunoglobulins from the dam to the neonate. The test involves precipitating high molecular weight proteins out of solution, which causes turbidity that is then assessed using a colourimeter. There is a clear correlation between low ZST results and poor health in calves, relating to both low *IgG* levels and low total immunoglobulin levels.

During 2018, 1156 blood samples (submitted by veterinary practitioners or collected at *post-mortem* examination of carcases at AFBI) were tested by AFBI. Of

Figure 17: Results of Zinc Sulphate Turbidity tests performed by AFBI in 2018 from submitted diagnostic bovine calf serum samples (n= 968). Adequate colostral immunity is defined as equal or greater than 20 *units*.

Violin Plot of ZST Test Results
Diagnostic submissions

Figure 18: Distribution and violin plot of results of ZST values in AFBI during 2018 from submitted diagnostic bovine calf serum samples. Adequate colostral immunity is defined as equal or greater than 20 *units* (orange line). Those cases greater than 60 *units* are not included.

Trichostrongyle eggs

Figure 19: Relative frequency of detection of trichostrongyle eggs in bovine faecal samples examined in AFBI in 2018 in relation to a commonly used threshold of significance- 500 eggs per gram (n=2280).

these 694 (60.03 *per cent*) returned a value of lower than 20 *units*, *i.e.* there had been inadequate passive transfer of immunoglobulins.

Further analysis shows that, of the 968 blood samples submitted by vets in practice, 555 (57.33 *per cent*) were inadequate (Figure 17) and of the 188 samples taken during a post mortem 139 (73.94 *per cent*) were inadequate (Figure 16), highlighting the link between calf mortality and inadequate passive transfer of immunoglobulins. Distribution of results of ZST values from submitted diagnostic bovine calf serum samples is plotted in a violin plot in Figure 18.

Gut closure in neonates begins almost immediately after birth, consequently calves need to get an adequate volume of colostrum, at least three litres, within the first two hours of life. Calves will acquire 90 *per cent* of their immunoglobulins through colostrum, therefore, correct colostrum management is essential.
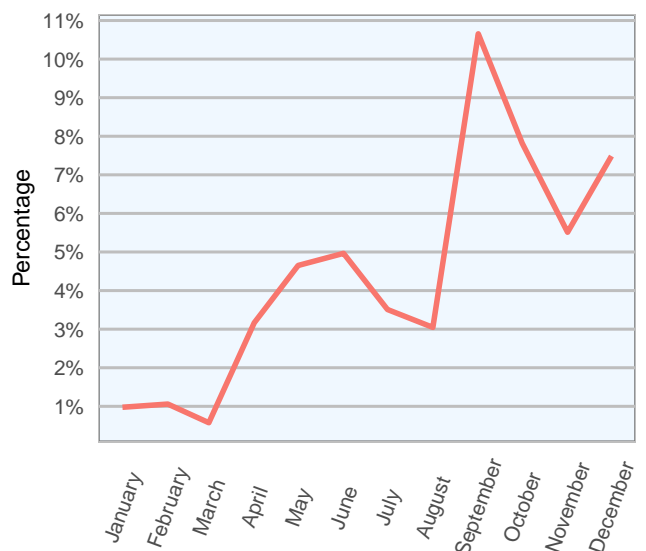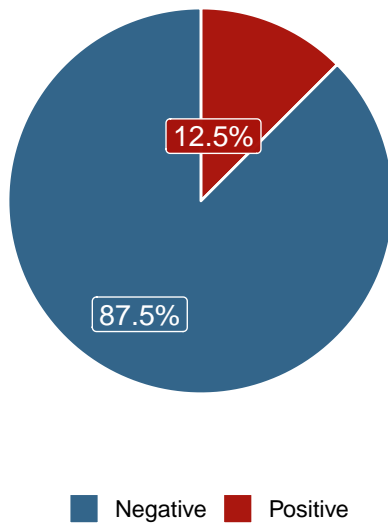
Bovine Parasites

Liver fluke. In 2018, *Fasciola hepatica* incidence dropped to 12.5 *per cent* of bovine faecal samples submitted to AFBI (Figure 21). Pathogenesis depends on the number of ingested metacercariae (immature fluke)

Trichostrongyle faecal egg counts

Figure 20: Monthly percentage of bovine faecal samples tested by AFBI in 2018 with a positive trichostrongyle egg count (positive samples have equal or greater to 500 eggs *per gram*).
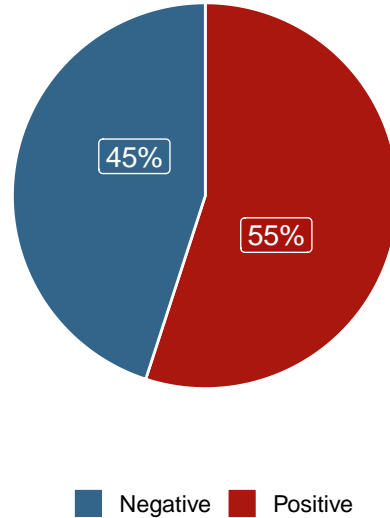
Liver fluke eggs

Figure 21: Relative frequency of detection of liver fluke eggs in bovine faecal samples examined in AFBI in 2018 (n=2001).

and stage of parasite development within the liver; first phase occurs while parasites migrate through hepatic parenchyma and the second while parasites reside in bile ducts, the latter leading to characteristic calcification of ducts and the *pipe-stem liver* appearance visible on *post-mortem* (Taylor *et al.*, 2015). Diagnosis is most common in late winter and early spring. Liver fluke infection, Fasciolosis, has major economic associations on livestock productivity due to the resulting morbidity and mortality (McCann *et al.*, 2010), with carcases that have been infected by liver fluke having poorer conformation and lower cold weight than those free of liver fluke (Sanchez-Vazquez and Lewis, 2013).
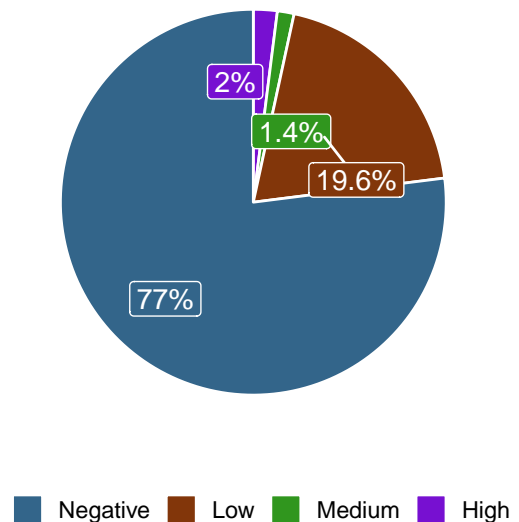
Rumen fluke. *Paramphistomum spp.* are found in the reticulum and rumen and are generally well tolerated, even with heavy burdens. Any pathogenic effect is usually associated with the intestinal phase of infection, where immature flukes attach to duodenal mucosa before migrating to forestomachs; diarrhoea, anorexia and rectal haemorrhage may be noted. However, a large number of animals with rumen fluke eggs detected in their faeces show few, if indeed any, clinical signs of disease. Incidence of positive bovine faecal samples remains similar to 2017, 55 *per cent* (Figure 22).

Rumen fluke eggs

Figure 22: Relative frequency of detection of rumen fluke eggs in bovine faecal samples examined in AFBI in 2018 (n=1998).

Coccidial oocysts

Figure 23: AFBI results for bovine faecal samples tested for coccidial oocysts during 2018 (n=2281).

Figure 24: An ovine kidney with haemoglobinuric nephrosis due to copper toxicity. Photo: Sean Fee.

Figure 25: The trachea of a lamb with *Bibersteinia trehalosi*. Photo: Sean Fee.

Coccidia spp.. Animals are infected by ingesting oocysts from contaminated pasture. Coccidiosis can cause significant economic losses to farmers due to reduced performance and mortality caused by heavy burden predominantly in younger animals. In 2018 there was a slight decrease in the percentage of positive samples, from 27 *per cent* in 2017 to 23 *per cent* in 2018 (Figure 23).

Ovine Diseases

Causes of sheep mortality in 2018. The number of sheep submissions in Northern Ireland decreased slightly in 2018 compared to 2017, 510 compare with 559 respectability (Table 11 and Figure 26).
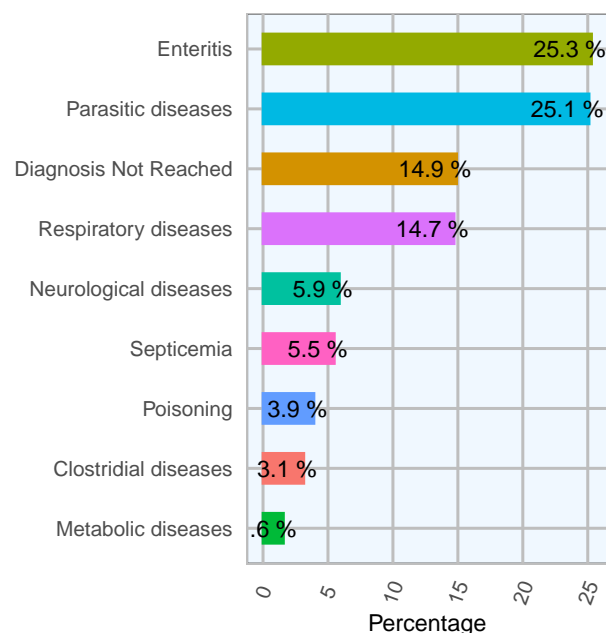
In 2018, enteritis, parasitic disease and respiratory disease were the most commonly diagnosed causes of death in sheep of all ages in Northern Ireland. The relative importance of clostridial diseases decreased slightly in 2018 compared to 2017, from 5.7 *per cent* to 3.1 *per cent*.

Parasitic Disease. Parasitic disease was the second most common diagnosis in sheep in 2018, 25.1 *per cent* of cases (Table 11). In the ovine faecal samples examined rumen fluke eggs were more frequently detected than liver fluke eggs (Figures 28 and 29), this is similar to previous years.

Ovine Abortions. Foetii from 333 ovine abortions and stillbirths were examined during 2018 in Northern Ireland (Table 12 and Figure 33), compared to 244 abortions examinations in 2017. Significant pathogens were
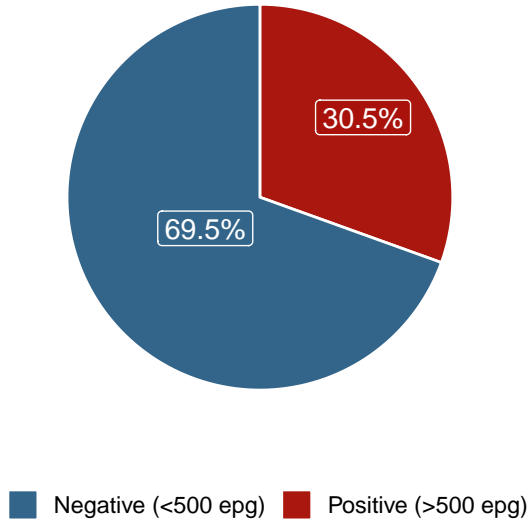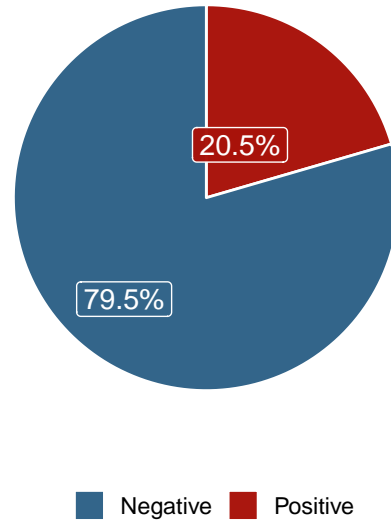

Figure 26: The conditions most frequently diagnosed on *post-mortem* examinations of ovine carcases in 2018 (n= 510).

CORRY S. & WALSH A

Trichostrongyle eggs (ovine samples)

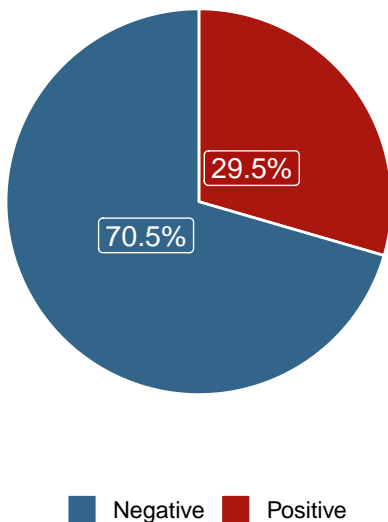Negative (<500 epg)　Positive (>500 epg)

Figure 27: Relative frequency of detection of trichostrongyle eggs in ovine faecal samples examined in AFBI in 2018 in relation to a commonly used threshold of significance- 500 eggs per gram (n=942).

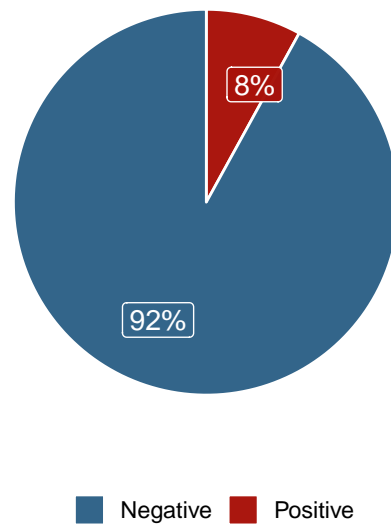## Rumen fluke eggs (ovine samples)

Negative　Positive

Figure 29: Relative frequency of detection of rumen fluke eggs in ovine faecal samples examined in AFBI in 2018 (n=829).

Liver fluke eggs (ovine samples)
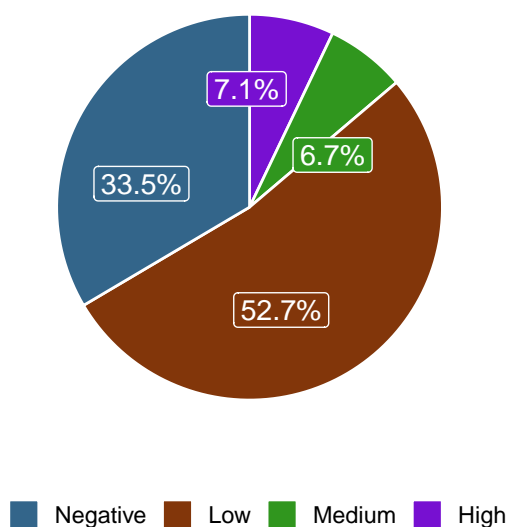

Negative　Positive

Figure 28: Relative frequency of detection of liver fluke eggs in ovine faecal samples examined in AFBI in 2018 (n=829).
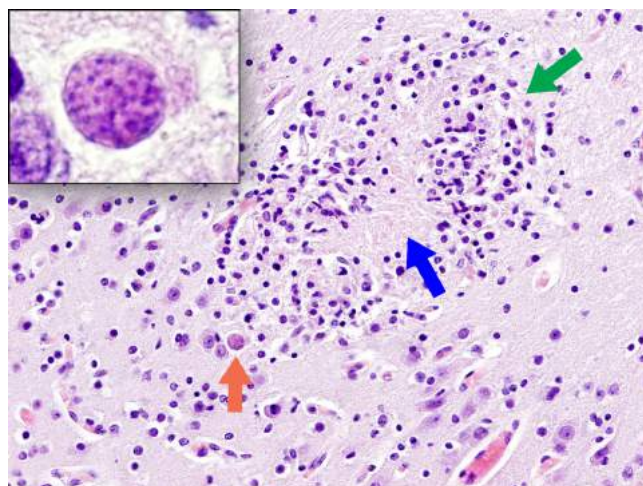
Nematodirus eggs (ovine samples)

Negative　Positive

Figure 30: Relative frequency of detection of Nematodirus eggs in ovine faecal samples examined in AFBI in 2018 (n=944).

Coccidial oocysts (ovine samples)

- ■ Negative
- ■ Low
- ■ Medium
- ■ High

Figure 31: AFBI results for ovine faecal samples tested for coccidial oocysts during 2018 (n=943).

Table 11: Conditions most frequently diagnosed on *post-mortem* examination of sheep by AFBI in 2018 (n= 510).

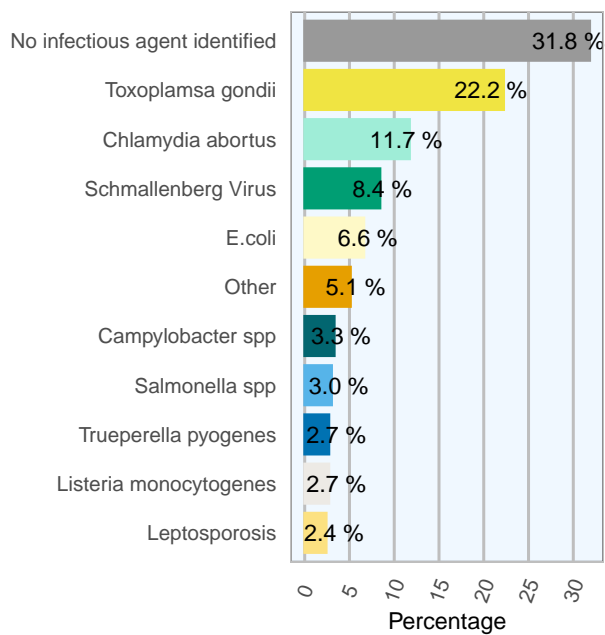| Category	No. of cases	Percentage
Enteritis	129	**25.3**
Parasitic diseases	128	**25.1**
Diagnosis Not Reached	76	**14.9**
Respiratory diseases	75	**14.7**
Neurological diseases	30	**5.9**
Septicemia	28	**5.5**
Poisoning	20	**3.9**
Clostridial diseases	16	**3.1**
Metabolic diseases	8	**1.6**

Table 12: Most frequently causes of ovine abortion diagnosed at AFBI in 2018 (n= 333).

Category	No. of cases	Percentage
No infectious agent identified	106	**31.8**
Toxoplamsa gondii	74	**22.2**
Chlamydia abortus	39	**11.7**
Schmallenberg Virus	28	**8.4**
E.coli	22	**6.6**
Other	17	**5.1**
Campylobacter spp	11	**3.3**
Salmonella spp	10	**3.0**
Trueperella pyogenes	9	**2.7**
Listeria monocytogenes	9	**2.7**
Leptosporosis	8	**2.4**

Figure 32: Characteristic histological lesion of *Toxoplasma gondii* encephalitis in the brain of a lamb foetus: focal necrosis (blue arrow) surrounded by a margin of reactive glial cells (green arrow). Note the presence of a tachyzoite (orange arrow and inset) adjacent to the lesion. Photo: Cosme Sánchez-Miguel.

detected in 210 cases (63 *per cent*). Pathogens identified included *Toxoplasma gondii* (74 cases, 22.2 *per cent*), *Chlamydophilia abortus* (39 cases, 11.7 *per cent*), *E. coli* (22 cases, 6.6 *per cent*), *Campylobacter spp.* (11 cases, 3.3 *per cent*), *Salmonella spp.* (10 cases, 3 *per cent*), *Trueperella pyogenes* (9 cases, 2.7 *per cent*) and *Listeria spp.* (9 cases, 2.7 *per cent*)*Leptospira spp.* (8 cases, 2.4 *per cent*).The percentages of these isolates are very similar to those in 2017.

Toxoplasma gonddi continues to be the most commonly diagnosed pathogen despite vaccination being widely available. Susceptible sheep are infected following ingestion of food or water contaminated with oocysts, which are highly resistant and can survive for long periods in moist conditions. Infection in early gestation can result in foetal death and resorption, whilst infection late in gestation can result in lambs being born normal and immune. However, infection mid gestation usually result in still born lambs, weak lambs and mummified foetuses. *Toxoplasma gondii* is usually isolated from typical white lesions in placental cotyledons and foetal tissues (Figure 32). Ewes that abort

Figure 33: The most frequently diagnosed causes of ovine abortion diagnosed at AFBI in 2018 (n= 333).

due to *Toxoplasma gondii,* usually lamb normally in subsequent years.

References

Buczinski S, Rezakhani A, Boerboom D (2010). "Heart disease in cattle: Diagnosis, therapeutic approaches and prognosis." *The Veterinary Journal*, **184**(3), 258 – 263. ISSN 1090-0233. doi: https://doi.org/10.1016/j.tvjl.2009.05.005. URL http://www.sciencedirect.com/science/article/pii/S1090023309001907.

Malmuthuge N, Guan LL (2017). "Understanding the gut microbiome of dairy calves: Opportunities to improve early-life gut health1." *Journal of Dairy Science*, **100**(7), 5996 – 6005. ISSN 0022-0302. doi:https://doi.org/10.3168/jds.2016-12239. URL http://www.sciencedirect.com/science/article/pii/S0022030217304046.

McCann CM, Baylis M, Williams DJ (2010). "The development of linear regression models using environmental variables to explain the spatial distribution of Fasciola hepatica infection in dairy herds in England and Wales." *International Journal for Parasitology*, **40**(9), 1021 – 1028. ISSN 0020-7519. doi: https://doi.org/10.1016/j.ijpara.2010.02.009. URL http://www.sciencedirect.com/science/article/pii/S0020751910000676.

Quinn PJ, Markey BK, Leonard FC, Fitzpatrick ES, Fanning S (2015). *Concise Review of Veterinary Microbiology*. Second edition. Wiley-Blackwell. URL https://www.wiley.com/en-us/Concise+Review+of+Veterinary+Microbiology%2C+2nd+Edition-p-9781118882700.

Sanchez-Vazquez MJ, Lewis FI (2013). "Investigating the impact of fasciolosis on cattle carcase performance." *Veterinary Parasitology*, **193**(1), 307 – 311. ISSN 0304-4017. doi: https://doi.org/10.1016/j.vetpar.2012.11.030. URL http://www.sciencedirect.com/science/article/pii/S0304401712006152.

Taylor MA, Coop RL, Wall R (2015). *Veterinary Parasitology*. Fourth edition edition. Wiley Blackwell. ISBN 9780470671627. URL https://blackwells.co.uk/bookshop/product/9780470671627?gC=8ad8757ba&gclid=EAIaIQobChMIg5nu1ca85AIVRLTtCh3aVQQzEAYYASABEgI-cfD_BwE.

R Packages Used In This Report

All-Island Animal Disease Surveillance Report 2018

Veterinary Laboratory Service

October 3, 2019

The data analysis, construction of graphics and visualisation of the 2018 Veterinary Laboratory Service data have been conducted by using the R programming language(R Core Team, 2013) and the RStudio integrated development environment. Eddelbuettel and Balamuta (2019), with their *pinp* package have provided the template for this report, which is originally based on the PNAS [1] article style. Most of the charts were plotted with the package *ggplot2* (Wickham *et al.*, 2018a) and the tables constructed with kableExtra (Zhu, 2018) and *finalfit* (Harrison *et al.*, 2019).

The webpage (HTML) version of the Disease Surveillance Report has also been compiled in RStudio by applying the package bookdown (Xie, 2018a). An extensive use of R Markdow and LaTeX languages were also utilised with the packages, *pinp* and *bookdown* for formatting and typesetting.

Many other packages were also used in the preparation of this report, as shown below.

References.

Allaire J, Xie Y, McPherson J, Luraschi J, Ushey K, Atkins A, Wickham H, Cheng J, Chang W, Iannone R (2018). *rmarkdown: Dynamic Documents for R*. R package version 1.11, URL https://CRAN.R-project.org/package=rmarkdown.

Appelhans T, Detsch F, Reudenbach C, Woellauer S (2018). *mapview: Interactive Viewing of Spatial Data in R*. R package version 2.6.0, URL https://CRAN.R-project.org/package=mapview.

Arnold JB (2018). *ggthemes: Extra Themes, Scales and Geoms for 'ggplot2'*. R package version 4.0.1, URL https://CRAN.R-project.org/package=ggthemes.

Attali D, Baker C (2018). *ggExtra: Add Marginal Histograms to 'ggplot2', and More 'ggplot2' Enhancements*. R package version 0.8, URL https://CRAN.R-project.org/package=ggExtra.

Barrowman N (2019). *vtree: Display Information About Nested Subsets of a Data Frame*. R package version 1.0.0, URL https://CRAN.R-project.org/package=vtree.

Bivand R, Lewin-Koh N (2018). *maptools: Tools for Handling Spatial Objects*. R package version 0.9-4, URL https://CRAN.R-project.org/package=maptools.

Bivand R, Rundel C (2018). *rgeos: Interface to Geometry Engine - Open Source ('GEOS')*. R package version 0.4-2, URL https://CRAN.R-project.org/package=rgeos.

Dahl DB, Scott D, Roosen C, Magnusson A, Swinton J (2018). *xtable: Export Tables to LaTeX or HTML*. R package version 1.8-3, URL https://CRAN.R-project.org/package=xtable.

Daróczi G, Tsegelskyi R (2018). *pander: An R 'Pandoc' Writer*. R package version 0.6.3, URL https://CRAN.R-project.org/package=pander.

Eddelbuettel D, Balamuta J (2019). *pinp: 'pinp' is not 'PNAS'*. R package version 0.0.7, URL https://CRAN.R-project.org/package=pinp.

Harrison E, Drake T, Ots R (2019). *finalfit: Quickly Create Elegant Regression Results Tables and Plots when Modelling*. R package version 0.9.0, URL https://CRAN.R-project.org/package=finalfit.

Kahle D, Wickham H (2016). *ggmap: Spatial Visualization with ggplot2*. R package version 2.6.1, URL https://CRAN.R-project.org/package=ggmap.

Neuwirth E (2014). *RColorBrewer: ColorBrewer Palettes*. R package version 1.1-2, URL https://CRAN.R-project.org/package=RColorBrewer.

Pebesma E (2018). *sf: Simple Features for R*. R package version 0.7-1, URL https://CRAN.R-project.org/package=sf.

Pebesma E, Bivand R (2018). *sp: Classes and Methods for Spatial Data*. R package version 1.3-1, URL https://CRAN.R-project.org/package=sp.

Powell C (2018). *CGPfunctions: Powell Miscellaneous Functions for Teaching and Learning Statistics*. R package version 0.4.1, URL https://github.com/ibecav/CGPfunctions.

R Core Team (2013). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

RStudio, Inc (2017). *htmltools: Tools for HTML*. R package version 0.3.6, URL https://CRAN.R-project.org/package=htmltools.

Slowikowski K (2018). *ggrepel: Automatically Position Non-Overlapping Text Labels with 'ggplot2'*. R package version 0.8.0, URL https://CRAN.R-project.org/package=ggrepel.

Spinu V, Grolemund G, Wickham H (2018). *lubridate: Make Dealing with Dates a Little Easier*. R package version 1.7.4, URL https://CRAN.R-project.org/package=lubridate.

Tantau T (2006). *The PGF/TikZ manual*. The PGF/TikZ manual, URL https://www.bu.edu/math/files/2013/08/tikzpgfmanual.pdf.

Wickham H (2017). *reshape2: Flexibly Reshape Data: A Reboot of the Reshape Package*. R package version 1.4.3, URL https://CRAN.R-project.org/package=reshape2.

Wickham H (2018a). *scales: Scale Functions for Visualization*. R package version 1.0.0, URL https://CRAN.R-project.org/package=scales.

Wickham H (2018b). *stringr: Simple, Consistent Wrappers for Common String Operations*. R package version 1.3.1, URL https://CRAN.R-project.org/package=stringr.

---

[1] Proceedings of the National Academy of Sciences of the United States of America

Wickham H, Bryan J (2018). *readxl: Read Excel Files*. R package version 1.1.0, URL https://CRAN.R-project.org/package=readxl.

Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K (2018a). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics*. R package version 3.1.0, URL https://CRAN.R-project.org/package=ggplot2.

Wickham H, François R, Henry L, Müller K (2018b). *dplyr: A Grammar of Data Manipulation*. R package version 0.7.8, URL https://CRAN.R-project.org/package=dplyr.

Wickham H, Henry L (2018). *tidyr: Easily Tidy Data with 'spread()' and 'gather()' Functions*. R package version 0.8.2, URL https://CRAN.R-project.org/package=tidyr.

Wickham H, Hester J, Francois R (2018c). *readr: Read Rectangular Text Data*. R package version 1.3.0, URL https://CRAN.R-project.org/package=readr.

Xie Y (2018a). *bookdown: Authoring Books and Technical Documents with R Markdown*. R package version 0.9, URL https://CRAN.R-project.org/package=bookdown.

Xie Y (2018b). *knitr: A General-Purpose Package for Dynamic Report Generation in R*. R package version 1.21, URL https://CRAN.R-project.org/package=knitr.

Zhu H (2018). *kableExtra: Construct Complex Table with 'kable' and Pipe Syntax*. R package version 0.9.0, URL https://CRAN.R-project.org/package=kableExtra.