

The unpredictable epidemiology of *Dictyocaulus viviparus* lungworm infection of cattle

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Together, helminth parasites are the most important animal health constraint to global ruminant livestock production. Better control of gastrointestinal and lungworm nematodes (and liver flukes) in Irish and UK cattle is a priority to meet the need for food security through efficient livestock production.

Persistent acting macrocyclic lactone anthelmintics are convenient to use and highly effective against the main gastrointestinal roundworm parasites of cattle, *Ostertagia ostertagi*. Most cattle farmers worldwide depend upon the use of macrocyclic lactone anthelmintics for the control of nematode parasites, creating a scenario whereby global ruminant livestock production would be untenable without these drugs.

Macrocyclic lactone anthelmintic treatment programmes aimed at the control of *O ostertagi* sometimes create opportunities for completion of the life cycles of other parasites, such as *Cooperia* spp and *Nematodirus helvetianus* gastrointestinal worms, and *Dictyocaulus viviparus* lungworms. Consequently, in many parts of Ireland and the UK, *D viviparus* has become the major parasitic roundworm challenge to cattle production.

PARASITIC BRONCHITIS CAUSED BY *D VIVIPARUS* LUNGWORMS

In recent years, there has been a significant increase in the incidence of parasitic bronchitis in adult cattle in the UK and continental Europe (David, 1999; Van Dijk, 2004). A similar pattern of an increasing prevalence of parasitic bronchitis among cows has also been observed in Irish dairy herds. This change in the age profile of affected hosts has a major impact on dairy herd health and profitability.

The nature and severity of lungworm disease depends on the number of larvae ingested and on the host's responses to the parasitic stages of *D viviparus*. *D viviparus* has both direct and indirect effects on its cattle hosts, the latter being a consequence of inflammatory responses, evoked by the parasites to create environments that provide nutrients required for reproduction and egg production. Individual cattle differ in the extent and severity of these innate and adaptive responses.

In the absence of intervention or control, parasitic bronchitis is primarily a disease of previously unexposed cattle. In these unusual circumstances, most disease outbreaks occur during the first grazing season, between August and October in Ireland and the UK. The clinical signs of respiratory disease include extension of the neck, open-



Figure 1: Lungworm is a major production limiting constraint to Irish dairy cattle.

mouthed breathing and a harsh cough, referred to as 'hoose' or 'husk'. Morbidity rates are usually high and weight loss can be dramatic. Some animals may die, while less severely affected cattle self cure over a period of several months (Matthews et al, 2008).

In practice, nematode control strategies aimed at suppressive management of *O ostertagi*, in particular those using persistent acting macrocyclic lactone anthelmintic regimes, prevent exposure of naïve cattle to *D viviparus* (Fischer and Jacobs, 1995). Hence, parasitic bronchitis only arises if challenge occurs once the drug persistence is ended. In these situations, the source of challenge may be L_3 released by rainfall from the faecal pats of untreated animals, or L_3 that have been wind-borne, previously dormant in the soil, or carried by earthworms and wildlife. The onset of primary infection causing parasitic bronchitis in naïve animals is, therefore, unpredictable, and often seen in older animals during their second, third or subsequent grazing seasons.

Grazing management can have similar effects on delaying first exposure to *D viviparus*, hence outbreaks of parasitic bronchitis commonly occur in spring-born suckler calves at the end of their first grazing season. This situation might arise because the dams remove overwintered *D viviparus* L_3 from herbage early in the season, minimising the challenge to the calves once they start to graze (Jacobs et al, 1985). The source of larval challenge at the end of the season might be L_3 released by rainfall from cow faecal pats deposited earlier in the season, or wind-borne L_3 .

Host protective immunity to *D viviparus* is short-lived and requires continuous or regular re-exposure (Michel and Mackenzie, 1965). Hence, outbreaks of parasitic bronchitis

are seen in previously exposed cattle, when challenged following a period of no exposure. The severity of these outbreaks depends upon the level of *D viviparus* L3 challenge, and the re-onset of the hosts' immune responses. Periods of no exposure may result from prolonged housing, grazing management, or use of macrocyclic lactone anthelmintic drugs. In recent years, this 're-infection syndrome' has become commonplace in adult dairy cattle, putatively correlating with the increased use of eprinomectin pour-on treatments of lactating animals for ectoparasite, gastrointestinal nematode and lungworm control (Höglund et al, 2003). Affected adult cattle typically show severe clinical signs of respiratory disease, dramatically reduced milk yields, weight loss and sometimes death (Eysker et al, 1994).

It is not unusual for primary infection and 're-infection syndrome' to occur simultaneously in the same herd, affecting heifers or second-calvers, and older cows, respectively. In these cases, patent infections arise in the younger, but not necessarily the older, animals, which may not be completely immunologically naïve. The older animals are generally most severely affected.

CATTLE LUNGWORM BIOLOGY AND LIFECYCLE

In common with the gastrointestinal trichostrongyle nematode parasites of cattle, *D viviparus* lungworms have direct lifecycles, alternating between free-living developing (L₁ and L₂) and infectious larvae (L₃), and parasitic larval (L₃ and L₄) and adult stages. Hence, the epidemiology of lungworm outbreaks in cattle is driven by influences of climatic conditions on the free-living stages and of host effects on the parasitic stages.

Adult *D viviparus* lungworms in the bronchi and trachea are dioecious and sexually reproducing, with ovo-viviparous

females producing more than 1,000 eggs per day. Eggs shed by females are coughed into the pharynx, hatched, then swallowed with mucus into the gastrointestinal tract. L₁ are voided into the environment in the faeces, where they undergo two moults to become L₃, retaining the cuticle of the L₂ as a protective sheath. L₁ and L₂ can feed on faecal bacteria, but are hatched with sufficient energy reserves that they may not require to do so. Sheathed L₃ cannot feed and must migrate from the faeces onto herbage, where they become the infective stage for their cattle hosts.

The rate of development of L₁ to infective L₃ is influenced by the temperature and moisture of the faeces in the environment. Larval feeding and development only occurs at temperatures above about 10°C, being optimal at about 20°C. At higher temperatures larvae develop faster, but have depleted energy reserves, hence, higher mortality rates. Larval development and survival only occurs at high levels of humidity, which may be afforded within the protected cattle faecal biome, even when environmental conditions are dry. Given optimal conditions, development from L₁ to L₃ may take as little as five days. However, the larvae are only released from the protective environment of the faeces and migrate onto herbage when environmental conditions are wet or humid.

The bionomics of lungworm infective larval challenge are complex and infection is often unpredictable. L₃ survival on herbage is finite and generally short, while desiccation is lethal. However, during dry periods, L₃ may be retained within the faecal pats, then released and dispersed onto herbage more-or-less en masse when rainfall next occurs, giving rise to sudden high levels of challenge. Some L₃ overwinter on pasture, depending on environmental conditions (most parasites survive overwinter as adults or inhibited L₅ in carrier animals). Others may survive for

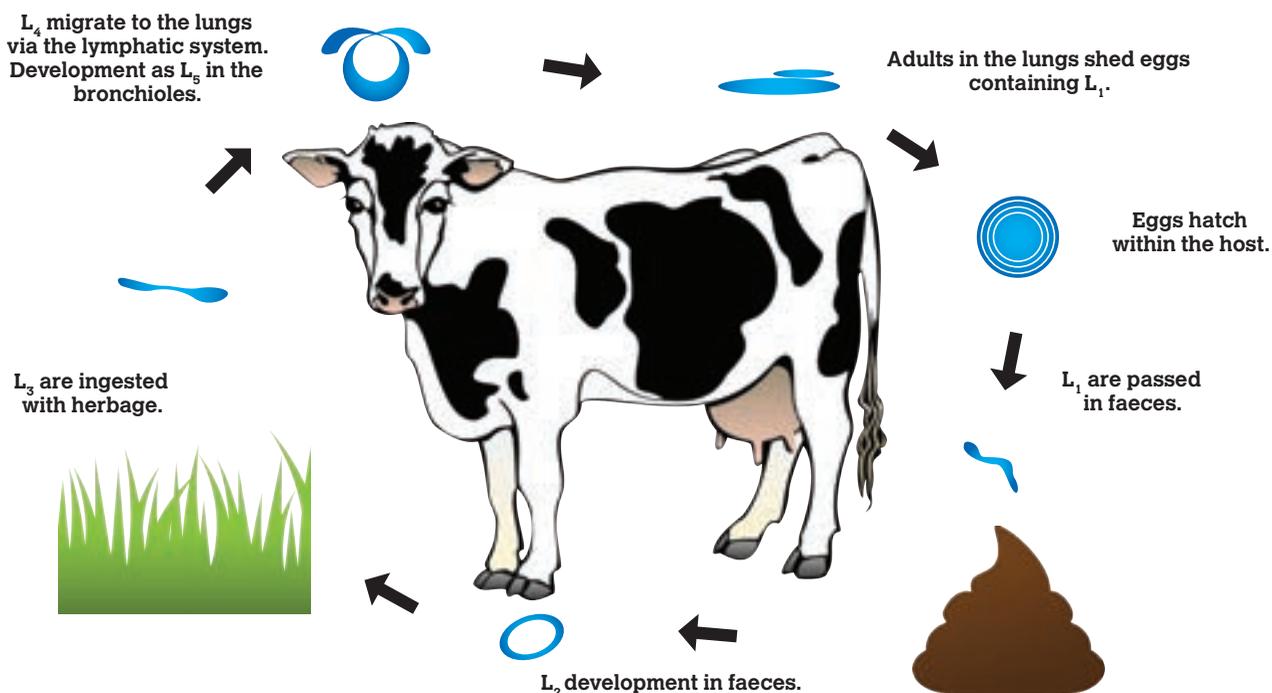


Figure 2: Lifecycle of *D viviparus*.



Figures 3a, 3b and 3c: Lungworm in the trachea. Photo: Donal Toolin, Kilkenny Regional Veterinary Laboratory.

prolonged periods in a state of dormancy in the soil. In warm and humid weather conditions, some L_3 may migrate onto the spore forming bodies of *Pilobolus* fungi growing on the faeces, from where they can become airborne and spread to other pastures (Eysker, 1991). L_3 may also be spread by earthworms, with manure, or by wildlife, hence prediction of pasture larval challenge is challenging. Outbreaks of parasitic bronchitis caused by *D viviparus* are commonly reported in animals grazed on pastures that have not been used by cattle during the previous year, or that have recently been cultivated and reseeded, where the risks of *O ostertagi* causing parasitic gastroenteritis would be very low.

The L_3 challenge to induce disease in cattle can be small. Ingested L_3 exsheath in the gastrointestinal tract of the host, then migrate through the intestinal wall to the mesenteric lymph nodes where they moult. L_4 migrate through the lymphatics and blood to the lungs, entering the alveoli and moulting once more to adults while moving up the respiratory tree through the bronchioles to the larger airways. The period between ingestion of infective larvae and egg shedding by adult parasites (the pre-patent period) is about 21 to 28 days.

HOST-PROTECTIVE IMMUNITY

Host-protective immunity to *D viviparus* develops more rapidly than to gastrointestinal nematodes in response to natural challenge (Michel, 1962). However, a higher frequency of natural boosting is required to maintain immunity to *D viviparus*.

Thus, scenarios involving evasive pasture management, climatic influences, housing and use of persistent acting anthelmintics, which lead to lack of exposure to *D viviparus* for prolonged periods, result in loss of protective immunity. These factors may then predispose to clinical disease following subsequent challenge, and undoubtedly account for the changing epidemiology and age pattern of parasitic bronchitis in Ireland and the UK. The economic consequences of disease occurring in older animals are potentially greater than those of primary disease in young stock.

DIAGNOSIS OF INFECTION

The diagnosis of parasitic bronchitis is based upon knowledge or implication of prior exposure to *D viviparus* in conjunction with the animals' grazing management and anthelmintic treatment histories. Clinical examination of

affective animals is helpful to differentiate lungworm from other primary causes of respiratory disease. For example, animals with parasitic bronchitis are often not pyrexemic, while the generalised distribution of a range of lung sounds heard on auscultation can be characteristic.

Post-mortem findings include lung consolidation adjacent to the large airways and the presence of tangled masses of 4-5cm long, slender white worms within the sectioned area, are pathognomonic. Cellular infiltration of the epithelium of the alveoli, bronchioles and bronchi, interstitial emphysema and pulmonary oedema can be identified histologically. Consolidation around the infected bronchi is caused by cellular responses to eggs and L_1 aspirated into the alveoli. Patent *D viviparus* infection of live animals can be shown by the identification of L_1 in faeces. A simple Baermann method can be used to allow L_1 to migrate out of a 10-20g faecal sample suspended in water, and then sediment to the base of a funnel from where they can be collected, examined microscopically, identified on the basis of their size, morphology and the characteristic presence of dark brown food granules within the intestinal cells, and enumerated. An enzyme linked immune-sorbent assay (ELISA) has been developed to detect *D viviparus* specific serum or milk antibodies. The ELISA is based on antigens extracted from L_5 or adult stages, and does not detect pre-patent infections, including responses to vaccination. Antibody responses are not detected until four to five weeks after initial challenge. The ELISA is better suited to the testing of large numbers of animals, and less suitable for the diagnosis of disease in individual animals. Positive group or bulk sample titres provide a good indication of recent exposure, but cannot be used to show the acquisition of protective immunity. Titres become negative within a few months of host elimination of *D viviparus*.

TREATMENT OF PARASITIC BRONCHITIS

Treatment of parasitic bronchitis is not straightforward, and the prognosis is frequently guarded. Secondary infection with a range of viral, mycoplasma and bacterial pathogens is usually present. Treatment therefore involves use of broad spectrum anthelmintics, antibiotics and anti-inflammatory drugs. *D viviparus* causes an immune-mediated pneumonia, hence corticosteroids are more effective than non-steroidal anti-inflammatory drugs, but cannot be used in animals in the third trimester of pregnancy.

It is sometimes stated that anthelmintic treatment of severe cases of parasitic bronchitis can exacerbate clinical



Figure 4: What are these animals eating? It is important to consider the source of lungworm infective larvae when considering disease control.

signs due to an allergic reaction to the presence of dead parasites in the lungs, and may even precipitate host death. Consequently, levamisole is often recommended as the anthelmintic of choice for the treatment of parasitic bronchitis, because it first paralyses the parasites, allowing them to be coughed up and expelled, rather than dying in situ.

PRINCIPLES OF CONTROL

The general aims of nematode control are to limit host challenge to a level which does not compromise performance or welfare while at the same time enabling the development of immunity. Sustainable control programmes in individual herds are based on the common-sense application of knowledge of the farming system and of the relationship between pasture contamination, the availability of infective larvae on pasture and the build-up of infection in animals. Control is then achieved by grazing animals on safe pasture, or by using anthelmintic drugs to suppress pasture larval contamination.

Anthelmintic drugs are relatively cheap and convenient to use and pharmaceutical control regimes in cattle are straightforward and highly effective, at least for the management of gastrointestinal nematodes. Consequently, most UK and Irish beef and milk producers rely on the use of anthelmintics for the control of gastrointestinal nematodes and lungworm, most preferring the convenience, broad spectrum of anti-parasitic activity, and long persistence of action of the macrocyclic lactone pour-ons. Furthermore, the nil milk withhold period of eprinomectin makes this the drug of choice in lactating dairy herds.

Dairy calves and autumn-born beef calves turned out for the first time in spring, without their dams, are usually given suppressive anthelmintic treatments during their first grazing season. Spring-born beef calves turned out alongside their dams are usually given a single treatment at first housing for the prevention of type II ostertagiosis, and suppressive treatments during the second grazing season. However, these regimes are often so successful in the control of gastrointestinal nematodes that calves have insufficient exposure to L_3 challenge to acquire protective

immunity and remain susceptible during the following grazing season; protective immunity to gastrointestinal nematodes being slower in onset than to *D viviparus*. Anthelmintic treatments are therefore routinely given to cattle in their second and third grazing seasons, further delaying the acquisition of protective immunity against gastrointestinal nematodes (Stafford and Coles, 1999). These regimes also prevent the boosting of protective responses to lungworms.

Precise anthelmintic treatment regimes are governed by the persistence of the drugs. For example: ivermectin injections at a dose rate of 0.2mg/kg, which persist for about three and four weeks against *O ostertagi* and *D viviparus*, respectively, are given three, eight and 13 weeks after turnout; 5mg/ml doramectin pour ons, which persist for about five and six weeks against *O ostertagi* and *D viviparus*, respectively, are given at turnout and about eight and 16 weeks later; while the subcutaneous depot formulation of 10% moxidectin, at a dose rate of 1mg/kg, which persists for about 17 weeks against both *O ostertagi* and *D viviparus* is given once at turnout. These regimes almost totally suppress egg output and L_3 contamination for the first half of the grazing season, and if the cattle are set stocked, then pastures remain safe from gastrointestinal nematode L_3 contamination for the remainder of the season. Hence, these strategies are highly effective against gastrointestinal nematodes. However, the same set stocked pastures do not necessarily remain free from soil or air-borne *D viviparus* L_3 challenge, providing opportunities for completion of the lungworm life cycle and giving rise to inconsistent and unpredictable outbreaks of parasitic bronchitis later in the season.

VACCINATION

The unpredictable nature of *D viviparus* challenge and parasitic bronchitis, coupled with the inevitable unsustainability of disease control depending mostly upon the use of one class of anthelmintic drug provides a compelling argument for use and development of vaccination regimes. In fact, a highly effective lungworm vaccine was developed in the 1950s at the Glasgow University Veterinary School as the first vaccine in the world against a parasitic infection, and brought to market more than 50 years ago. Millions of cattle have since been vaccinated and effectively protected against lungworm infection. However, vaccine use has declined over the past two decades, as producers have become increasingly dependent upon macrocyclic lactone anthelmintic treatments.

Each vaccine dose contains 1,000 to 2,000 viable gamma irradiated *D viviparus* L_3 , which are administered orally to mimic natural infection, without completing their life cycle and causing clinical disease. According to the conventional data sheet recommendations, calves over two months old are given two doses, separated by four weeks, with the second dose given before turnout to grass. The vaccine can be safely used in adult and lactating cattle. Vaccination gives rapid-onset immunity, but requires natural



Figure 5: Parasitic disease is an inevitable consequence of long grazing seasons and high stocking densities that are needed to allow efficient Irish pastoral dairy production.

boosting (Michel and Shand, 1955). A single booster dose prior to turnout is recommended to boost immunity in circumstances where exposure to natural challenge may not have occurred during the previous grazing season. Care must be taken not to kill the vaccine L_3 , or prevent boosting of immunity by the use of suppressive anthelmintic treatment regimes for the control of gastrointestinal nematodes. There is a good argument to be made for the use of pulse release oxfendazole boluses, or repeated

anthelmintic treatments at intervals greater than their period of persistence; potentially allowing sufficient exposure between pulses or treatments to maintain immunity against both gastrointestinal nematodes and lungworms (Jacobs et al, 1987). When infection is present, vaccinated animals can still contribute to pasture contamination, so all animals in a group must be vaccinated. Calves with or recovering from viral, mycoplasma or bacterial pneumonia cannot be vaccinated.

Helminth parasites have large genomes, with large numbers of genes and high levels of polymorphism. For example, the latest high-quality assembly of the model parasitic nematode, *Haemonchus contortus*, genome is 350Mb with about 22,000 protein coding genes (Laing et al, 2013). Parasitic nematodes, not least *D viviparus*, will therefore inevitably evolve in response to both favourable and unfavourable conditions, afforded by effects of climatic or animal management changes on free-living stages and exposure of parasitic stages to anthelmintic drugs, respectively. Thus, in forthcoming years, changes in the epidemiology and clinical impact of parasitic bronchitis should be expected, while failure of drugs to control *D viviparus* will be inevitable.

In Irish grass-based production systems, most farms will continue to use anthelmintics strategically during the first grazing season to maximise growth rates. Due to the potential lack of exposure to lungworm larvae in the first grazing season these herds can be vaccinated with two doses of Bovilis Huskvac (MSD Animal Health) at six and two weeks before turnout in the second grazing season. It is also possible to vaccinate adult cows in herds where outbreaks of lungworm have occurred. Before the following grazing season, whole herd vaccination can be used to re-establish immunity to lungworm. Strategic use of anthelmintics in first lactation cows can be used during the grazing season.

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