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SHORT COMMUNICATION

Inefficacy of ivermectin and moxidectin treatments against *Dictyocaulus viviparus* in dairy calves

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Abstract
Background: The bovine lungworm *Dictyocaulus viviparus* negatively impacts bovine health and leads to substantial economic losses. Lungworm infections can be difficult to manage due to the unpredictable and severe nature of clinical outbreaks. Despite the widespread use of macrocyclic lactones (MLs) in grazing cattle in the UK, there have been no confirmed reports of resistant lungworms to date, with only one case of anthelmintic-resistant (ML) lungworm confirmed worldwide.
Methods: Lungworm Baermann filtrations were conducted on first-season grazing dairy calves as part of a wider study investigating anthelmintic resistance in gastrointestinal nematodes in Scotland using the faecal egg count reduction test.
Results: Clinical signs and significant numbers of lungworm larvae in faeces were observed after treatment with either ivermectin or moxidectin.
Limitations: There are no established guidelines for the diagnosis of resistant lungworms in the field. Currently, resistance can only be diagnosed after a controlled efficacy test has been conducted. This limits the conclusions that can be drawn; however, they are highly suggestive of resistance.
Conclusion: This short report describes the inefficacy of ivermectin and moxidectin against *D. viviparus* and is highly suggestive of ML resistance.

INTRODUCTION

Parasitic bronchitis is caused by the bovine lungworm *Dictyocaulus viviparus*. Lungworm infections can cause mild to severe respiratory distress, inappetence and, in severe cases, death.[1,2] *Dictyocaulus viviparus* is regarded as one of the most pathogenic endoparasites of cattle in the UK and is of severe welfare and economic concern. When exposed to a high level of challenge, youngstock with limited immunity are at significant risk of clinical disease.[3] In the UK, lungworm outbreaks have been increasing since 2009 and are most pronounced in Scotland and northern England, with a shift in seasonality from late summer/autumn to any time of year and a wider temporal distribution of reported clinical cases, although there is still a peak incidence in September.[4]

Despite the availability of a vaccine (Bovilis Huskvac, MSD Animal Health), there has been a decrease in its use since the advent of anthelmintic products belonging to the macrocyclic lactone (ML) class.[5,6] Reliance on a single anthelmintic class would be expected to select for resistance, as has rapidly occurred in gastrointestinal nematode (GIN) populations.[7] However, to our knowledge, only one case of confirmed resistance has been reported worldwide,[8,9] although two reports of lack of efficacy of eprinomectin in lactating dairy cows were reported in 2018 and 2020.[8,10] This short report describes the inefficacy of ivermectin and moxidectin treatment against *D. viviparus* and is highly suggestive of ML resistance.

MATERIALS AND METHODS

Farm information

The study was undertaken on a commercial dairy unit located in central Scotland. The region has average annual maximum/minimum temperatures of 12.7°C/5.7°C and an average annual rainfall of 1370.2 mm over 181.2 days. The monthly meteorological data for 2023 varied appreciably from those of the previous 30 years. On average, June was 3.2°C warmer, experienced 13.7 mm less rainfall and 87.8 more sunshine hours. On average, July was 0.4°C warmer, experienced 39.6 mm more rainfall and 10.9 fewer sunshine hours (Table S1).

The herd comprised 170 Holstein-Friesian milking cows and followers, totalling 400 individuals; no other stock was grazed on the holding. The herd had been closed for more than 10 years, with service by artificial insemination and an all-year-round calving pattern. Dairy X Beef male calves were reared and sold to slaughter at 24–32 months of age, with dairy heifers retained as replacements. The farm was recruited as part of a study investigating anthelmintic inefficacy in GIN using the faecal egg count reduction test (FECRT).

Study design

Thirty-three spring-born Holstein-Friesian heifer and Dairy X Beef first grazing season (FGS) calves were turned out onto 4.4 ha of permanent pasture on 2 May 2023 at a stocking density of 7.5 individuals/ha. The pasture had not been previously grazed that year and was only used for turnout of FGS calves. The calves are usually set-stocked from turnout in late April/early May until mid-September and then moved to new grazing until housing in mid-October. For seven years prior to the 2023 grazing season, all FGS calves were treated with moxidectin (Cydectin 10% LA Solution; Zoetis) at turnout and ivermectin (Enovex 0.5%, w/v, Pour-on Solution; Norbrook Laboratories) at housing. In previous years, calves were reported to have maintained good body condition and growth rates, there were no confirmed diagnoses of parasitic bronchitis on the farm and lungworms were not a significant concern to the farmer.

The 33 calves were not wormed at turnout, and faecal egg counts (FEC) and body conditions were monitored fortnightly from early July 2023 until the FEC reached a group average of ~100 eggs per gram (epg). In addition, opportunistic faecal samples were collected for lungworm detection using a modified Baermann filtration technique with a reported sensitivity of one patent female adult.[11] Freshly voided faeces were collected from pasture for this monitoring phase and Baermann filtrations were performed within 4 hours of collection by adding 30 g of faecal material to a 12-ply gauze, forming a pouch and suspending it in a 500 mL glass beaker of tepid water overnight. The following morning, the faecal material and most of the suspension were removed without disturbing the sediment. The sediment and washings were added to a Petri dish, and larvae were identified under a stereo microscope at 10× magnification (see Figure 1). High larvae counts were estimated by evenly distributing the sediment onto a scored Petri dish and counting the larvae present in one-quarter of its surface area. Multiple individuals were confirmed as *D. viviparus* by capillary sequencing of the ITS2 region using generic strongyle primers.[12]

FIGURE 1 *Dictyocaulus viviparus* L1 recovered by Baermann filtration of a per-rectum faecal sample collected after moxidectin treatment

On 21 July 2023, the group mean FEC reached 94 epg (range 68–178 epg). Fourteen days later (day 0 in the FECRT), half of the group (16 individuals) were treated with moxidectin (Cydectin 10% LA Solution for Injection; Zoetis) by subcutaneous ear injection and the other half (17 individuals) were treated with subcutaneous ivermectin (IVOMEC Classic Injection for Cattle and Sheep; Boehringer Ingelheim) at the manufacturers' recommended dose rates of 1.0 and 0.2 mg/kg bodyweight, respectively. Individual animal weight was estimated by dairy calf weight band (AHDB) according to the manufacturer's instructions with the calves' weight ranging from 190 to 230 kg. All dose calculations and administration of the anthelmintics were undertaken by the researchers. Individual per-rectum samples were collected from all animals for FEC on days 0 and 15. From the post-treatment groups, nine samples per group were randomly selected and processed for Baermann filtration.

RESULTS

Fifteen Baermann filtrations were performed on a random subset of samples collected 21 and 14 days prior to the FECRT (faeces collected from pasture), and no larvae were detected. On these visits, the calves were in good body condition and displayed no clinical signs of parasitic bronchitis or gastroenteritis. On the day of treatment, the calves were in good body condition and displayed no clinical signs, and the farmer was happy with their performance.

On day 15 post-treatment, several calves displayed clinical signs of parasitic bronchitis: intermittent coughing with increased frequency after they were moved to the holding pen. The farmer noted that some of the calves had begun to develop diarrhoea and that the group was not as 'bright'. Of the 18 per-rectum samples that were chosen for Baermann

TABLE 1 *Dictyocaulus viviparus* larvae counts of per-rectum samples collected 15 days after ivermectin or moxidectin treatment

Ivermectin		Moxidectin	
Animal ID	**Larvae counted**	**Animal ID**	**Larvae counted**
1	40	10	2
2	340[a]	11	2
3	12	12	300[a]
4	250[a]	13	210[a]
5	16	14	0
6	33	15	0
7	2	16	0
8	51	17	0
9	0	18	0

[a]Estimated larvae count per 30 g faeces.

analysis, 12 were positive for lungworm: eight from the ivermectin treatment group and four from the moxidectin treatment group (Table 1). The larval counts of the lungworm-positive individuals varied greatly, ranging from 2 to 340.

Following the identification of lungworm larvae in both ivermectin- and moxidectin-treated groups, the case was reported to the Veterinary Medicine Directorate and relevant pharmaceutical companies as an adverse event with a suspected lack of efficacy. All calves were treated with levamisole (Levacide Low Volume 7.5% Oral-Solution; Norbrook Laboratories) and a supportive treatment of flunixin (Finadyne 50 mg/mL Solution for Injection; MSD Animal Health). They were moved from the high-risk pasture to low-risk grazing: silage aftermath that heifers had grazed. Fourteen days after levamisole treatment, a Baermann filtration was conducted on faecal samples from 10 randomly selected individuals, and no larvae were recovered. The calves had maintained good body condition, although a minority still coughed intermittently.

DISCUSSION

MLs are active against both larval and adult *D. viviparus,* with an efficacy of more than 99% in susceptible populations.[13,14] The claimed period of protection from reinfection by lungworms is 28 and 120 days for IVOMEC and Cydectin 10% LA, respectively. Therefore, the larvae that were recovered on day 15 post-treatment were well within this period of activity. The expected speed of action of MLs against *D. viviparus* in order to eliminate adult worms from the lungs is estimated to be ~24 hours,[15] so the presence of larvae indicates the presence of patent adults. With a pre-patent period of 21–28 days, the females producing these larvae must have been present at some early developmental stage during the time of treatment. We therefore conclude that both treatments were ineffective, with the findings highly suggestive of an ML-resistant population of *D. viviparus.*

Although there are established guidelines for the detection of anthelmintic resistance in GIN based on the FECRT,[16,17] there are currently no guidelines for diagnosing resistance in *D. viviparus.* In the present study, the lack of a pre-treatment (day 0) larvae count meant that a larvae count reduction could not be conducted; however, the feasibility of calculating a reliable percentage reduction in this species is unclear.

We hypothesised that this selection of an ML-resistant *D. viviparus* population arose from repeated and prolonged exposure to moxidectin over multiple years. As this field was only used for FGS calves, which were always treated with a long-acting moxidectin product at turnout, refugia for lungworms would be severely limited. Furthermore, the long half-life of moxidectin provides an extended 'tail' where the drug remains in the host but at a sub-therapeutic concentration[18]; this period and eventual end of protection from reinfection likely coincided with increasing lungworm challenge in late autumn on this farm. In GIN, moxidectin resistance confers high-level cross-resistance to ivermectin,[19] and while the lungworm larval counts reported here are consistent with this finding, we are limited in the conclusions that can be drawn from this small study where ivermectin was also used at housing. The FECRT results for GIN infections in the same calves, collected as part of a wider study, identified both ivermectin and moxidectin resistance (88.9% efficacy [lower confidence interval (LCI) = 86.7%, higher confidence interval (HCI) = 92.2%] and 92.8% efficacy [LCI = 91.5%, HCI = 94%] respectively), again suggesting that continued reliance on MLs is not sustainable.

The previous climatic conditions may go some way to explain why a patent lungworm infection was not detected on this farm prior to the FECRT, but bovine lungworm epidemiology is relatively poorly understood. During the 2 months prior to the FECRT, climate conditions differed from those of the 30-year average; June was significantly warmer and drier, whereas July was wetter. It is hypothesised that these dry conditions would have been detrimental to the survival of any free-living nematodes on pasture and would have limited dispersal from the pat.[20] However, the humid conditions of July would have promoted the dispersal of larvae that would have accumulated during this period while also promoting survival.[20] Given this sequence of events, the sudden onset of disease in mid-August fits nicely with clinical signs expected to develop 22–26 days after the expected high parasite abundance on pasture.[4]

The prolonged period of protection offered by MLs, especially long-acting products, coupled with their ease of application, make them a popular choice for controlling lungworms and GIN in calves. However, it is imperative that producers and veterinary practitioners consider the possibility of anthelmintic resistance in lungworms and implement sustainable parasite control strategies.[21] In particular, vaccination against lungworm infection in calves can be used successfully alongside other management strategies to reduce anthelmintic usage.

AUTHOR CONTRIBUTIONS

Conceptualisation, methodology, investigation, resources, writing—original draft, writing—review and editing and project administration: Paul Campbell. *Conceptualisation, methodology, investigation, writing—original draft, writing—review and editing and supervision*: Andrew Forbes and Jennifer McIntyre. *Methodology and investigation*: Taylor Bartoschek and Kayleigh Devine. *Conceptualisation, methodology and investigation*: Kerry O'Neill. *Conceptualisation, methodology, investigation, resources, writing—original draft, writing—review and editing, project administration, supervision and funding acquisition*: Roz Laing and Kathryn Ellis.

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CONFLICT OF INTEREST STATEMENT

The authors report that they have no conflicts of interest. The funders had no involvement in the design, collection, analysis and interpretation of the data or in the writing or submission of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supporting Information of this article.

ETHICS STATEMENT

All research procedures as described were approved by the University of Glasgow MVLS College Ethics Committee (project no. 200210097).

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REFERENCES

1. Forbes A. Lungworm in cattle: epidemiology, pathology and immunobiology. Livestock. 2018;23(2):59–66.
2. May K, Brügemann K, König S, Strube C. The effect of patent *Dictyocaulus viviparus* (re)infections on individual milk yield and milk quality in pastured dairy cows and correlation with clinical signs. Parasit Vectors. 2018;11(1):24.
3. Morgan ER. Lungworm in cattle: a true survivor. Vet Rec. 2020;186(19):639–41.
4. McCarthy C, van Dijk J. Spatiotemporal trends in cattle lungworm disease (*Dictyocaulus viviparus*) in Great Britain from 1975 to 2014. Vet Rec. 2020;186(19):642.
5. Bain RK. Irradiated vaccines for helminth control in livestock. Int J Parasitol. 1999;29(1):185–91.
6. van Dijk J. The epidemiology and control of dictyocaulosis in cattle. Cattle Practice. 2004;12(2):133–45.
7. Kaplan RM. Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. Vet Clin North Am Food Anim Pract. 2020;36(1):17–30.
8. Jewell N, Jones J, Mitchell S, Marsman A. Treatment failure of lungworm in cattle. Vet Rec. 2019;185(12):379–80.
9. Molento MB, Depner RA, Mello MHA. Suppressive treatment of abamectin against *Dictyocaulus viviparus* and the occurrence of resistance in first-grazing-season calves. Vet Parasitol. 2006;141(3):373–76.
10. Animal & Plant Health Agency. Lungworm in dairy cows with suspected failure of previous pour-on eprinomectin treatment. GB cattle quarterly report. Disease surveillance and emerging threats. Vol. 27 (Q4: October–December 2020). p. 14–15.
11. Eysker M. The sensitivity of the Baermann method for the diagnosis of primary *Dictyocaulus viviparus* infections in calves. Vet Parasitol. 1997;69(1):89–93.
12. Bisset SA, Knight JS, Bouchet CLG. A multiplex PCR-based method to identify strongylid parasite larvae recovered from ovine faecal cultures and/or pasture samples. Vet Parasitol. 2014;200(1–2):117–27.
13. Benz GW, Roncalli RA, Gross SJ. Use of ivermectin in cattle, sheep, goats, and swine. In: Campbell WC, editor. Ivermectin and abamectin. New York, NY: Springer; 1989; 215–29.
14. Rehbein S, Visser M, Kellermann M, Letendre L. Reevaluation of efficacy against nematode parasites and pharmacokinetics of topical eprinomectin in cattle. Parasitol Res. 2012;111(3):1343–47.
15. Forbes A. Lungworm in cattle: treatment and control. Livestock. 2018;23(3):102–6.
16. Kaplan RM, Denwood MJ, Nielsen MK, Thamsborg SM, Torgerson PR, Gilleard JS, et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. Vet Parasitol. 2023;318:109936.
17. Geurden T, Smith ER, Vercruysse J, Yazwinski T, Settje T, Nielsen MK. World association for the advancement of veterinary parasitology (WAAVP) guideline for the evaluation of the efficacy of anthelmintics in food-producing and companion animals: general guidelines. Vet Parasitol. 2022;304:109698.
18. Le Jambre LF, Dobson RJ, Lenane IJ, Barnes EH. Selection for anthelmintic resistance by macrocyclic lactones in *Haemonchus contortus*. Int J Parasitol. 1999;29(7):1101–11.
19. Kaplan RM, Vidyashankar AN, Howell SB, Neiss JM, Williamson LH, Terrill TH. A novel approach for combining the use of in vitro and in vivo data to measure and detect emerging moxidectin resistance in gastrointestinal nematodes of goats. Int J Parasitol. 2007;37(7):795–804.
20. Rose JH. The bionomics of the free-living larvae of *Dictyocaulus viviparus*. J Comp Pathol Therap. 1956;66:228–40.
21. COWS. COWS—Control of Worms Sustainably [Internet]. 2023. Available from: https://www.cattleparasites.org.uk/

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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