

## Monitoring gastrointestinal nematode and liver fluke infections in Belgium by bulk tank milk ELISA: are we making progress in parasite control?

*De monitoring van maagdarmworm- en leverbotinfecties op Belgische melkveebedrijven met tankmelk ELISA's: maken we vooruitgang in parasietencontrole?*

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### ABSTRACT

Parasitic infections with gastrointestinal (GI) nematodes and liver fluke (*Fasciola hepatica*) are common causes of reduced productivity in ruminants. In this paper, the results of a voluntary monitoring campaign on helminth infections in Belgian dairy herds are summarized and discussed. From 2009 until 2011, a bulk tank milk sample was collected in autumn and analyzed by antibody-detecting bulk tank milk ELISA's to assess the level of exposure to GI nematodes and liver fluke. The number of farms participating in the survey increased over the years, from 1216 in 2009 to 1731 in 2011. The proportion of herds with high exposure to GI nematodes varied significantly between the three years, from 67% in 2009 over 70% in 2010 to 63% in 2011. The proportion of herds with high exposure to *F. hepatica* remained stable around 30%. Important regional variations were observed with levels of exposure to GI nematodes increasing from the north to the south of the country, whereas the distribution of *F. hepatica* was concentrated in the province of West-Flanders, the southern part of East-Flanders, Hainaut and the northern part of Luxembourg. It can be concluded that, when compared with surveys conducted in surrounding countries, the levels of helminth exposure in Belgium can be considered high, especially for GI nematodes. If the aim is to decrease levels of exposure in the future, this will likely require control methods based on altered timings of treatment and changes in pasture management.

### SAMENVATTING

Infecties met maagdarmnematoden en leverbot (*Fasciola hepatica*) zijn een belangrijke oorzaak van verminderde productiviteit bij herkauwers. In dit artikel worden de resultaten samengevat van een monitoringcampagne van helminthinfecties op Belgische melkveebedrijven. In het najaar van 2009 tot en met 2011 werden tankmelkstalen verzameld en onderzocht aan de hand van antistof-ELISA's voor het bepalen van de mate van blootstelling van de melkveebedrijven aan maagdarmwormen en leverbot. Het aantal deelnemende bedrijven in de campagne steeg van 1216 in 2009 tot 1731 in 2011. Het aantal bedrijven met een hoge mate van blootstelling aan maagdarmwormen vertoonde een significante variatie tussen de jaren en bedroeg 67%, 70% en 63% in de drie opeenvolgende jaren. Het aantal bedrijven met een hoge mate van blootstelling aan *F. hepatica* bleef stabiel rond 30%. Er waren aanzienlijke regionale verschillen in de resultaten. De mate van blootstelling aan maagdarmwormen vertoonde een duidelijke toename van het noorden naar het zuiden van het land. De distributie van *F. hepatica* was geconcentreerd in de provincie West-Vlaanderen, het zuidelijke gedeelte van Oost-Vlaanderen, Henegouwen en het noordelijke gedeelte van Luxemburg. Wanneer de resultaten van deze campagne vergeleken worden met de resultaten van soortgelijke enquêtes in de omliggende landen, kan besloten worden dat de Belgische melkveebedrijven een hoge mate van blootstelling aan maagdarmwormen vertonen. Als men deze mate van blootstelling wilt verlagen, moeten waarschijnlijk aanpassingen in het weidebeheer doorgevoerd worden en meer gebruik gemaakt worden van preventieve ontwormingsschema's.

### INTRODUCTION

Parasitic infections with gastrointestinal (GI) nematodes (mainly *Ostertagia ostertagi*) and liver

fluke (*Fasciola hepatica*) are an important cause of production losses in dairy cattle (Corwin, 1997; Kaplan, 2001). Production losses mainly consist of the reduction in milk production, but also the growth,

fertility and general condition of the animals can be affected. Recently, the annual cost of GI nematodes and liver fluke infections in the Flemish dairy sector has been estimated at € 10 million and € 8 million, respectively (Charlier et al., 2009).

Pasture management and the use of anthelmintics are the two cornerstones in the control of these infections, and they are widely applied to reduce the associated production losses (Bennema et al., 2010). Since a number of years, novel diagnostic tools based on the quantification of antibodies against *O. ostertagi* and *F. hepatica* in milk have become available. The application of these diagnostics on bulk tank milk provides a low cost, complimentary tool to assess the levels of parasitic exposure and target control efforts. Since 2009, a yearly voluntary monitoring campaign has been organized in Belgium, based on the analysis of bulk tank milk samples in autumn as a tool to estimate levels of helminth exposure and associated production losses. In this paper, the results of this monitoring campaign are summarized, with a focus on the most recent results (2011), and potential time trends are considered by comparing the results with the data of 2009 and 2010. Finally, the results are discussed with the aim to advance effective parasite control practices in Belgium.

## MATERIALS AND METHODS

### Selection of farms and sample collection

From 2009 till 2011, the Belgian veterinarians were yearly contacted by Merial Belgium to monitor the parasitic infection status on their clientele's farms. Subsequently, interested veterinarians contacted farmers within their clientele, and upon agreement by the farmer, a bulk tank milk sample was collected in October-November for the quantification of anti-parasitic antibodies. In Flanders, the samples were collected and analyzed by MCC Vlaanderen (Lier) and reported by DGZ Vlaanderen (Torhout). In Wallonia, the samples were taken on farm by the veterinarian, and shipped to ARSIA (Loncin), where the analysis of the samples and the reporting of the results were done.

### Bulk tank milk ELISA's and interpretation of the results

Antibodies against GI nematodes were assessed by the Svanovir® *O. ostertagi*-AB ELISA (Boehringer Ingelheim Svanova, Uppsala, Sweden). Higher test results (expressed as ODR) indicate higher levels of exposure to GI nematodes and higher production losses. In general, values < 0.5 ODR indicate low levels of exposure to GI nematodes and no parasite-induced production losses; whereas values > 0.8 ODR indicate high levels of exposure with important production losses (Forbes et al., 2008).

Antibodies against *F. hepatica* were assessed by an ELISA developed at Ghent University that detects

antibodies against the excretory-secretory products of *F. hepatica* (Charlier et al., 2007a; 2008) in Flanders or the Fasciolosis Verification Test (Institut Pourquier, Montpellier, France) (Reichel et al., 2005) in Wallonia. For the UGhent *F. hepatica* ELISA, values < 0.3 ODR indicate absence of *F. hepatica* infection; values 0.3 - 0.8 ODR represent an increased probability of exposure to *F. hepatica*, however, without the induction of important production losses; values > 0.8 ODR indicate a high probability of infection with *F. hepatica* with important production losses (Charlier et al., 2007a). For the Fasciolosis Verification Test, test results are interpreted as no or very weak infestation ( $S/P \leq 30$ ), low infestation ( $30 < S/P \leq 80$ ; less than 20% of herd infected) or medium/high infestation ( $S/P > 80$ ; more than 20% of herd infected) according to the manufacturer's manual.

### Data analysis

Apparent between-year differences in the proportion of herds with high levels of exposure to GI nematodes or *F. hepatica* were tested for statistical significance by a Chi-square test.

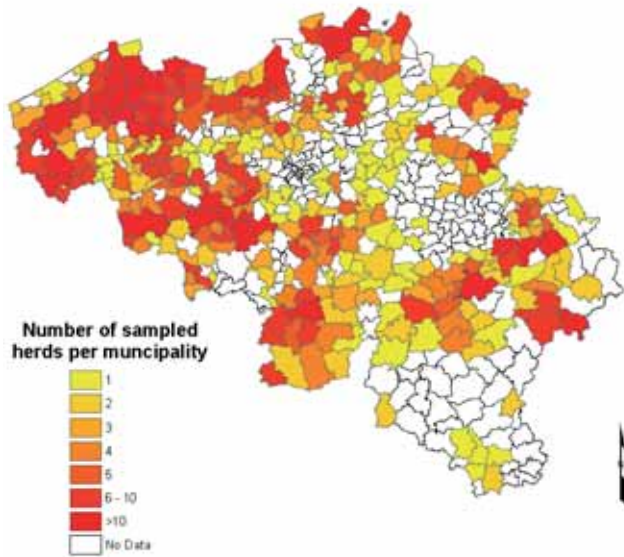
Maps representing the geographical distribution of the results of the monitoring campaign of 2011 were created in ArcGIS (ESRI, Redlands, USA). The data of 2011 were arranged per municipality, and joined with municipality maps of Belgium obtained from the National Geographical Institute (NGI) and the Agentschap voor Geografische Informatie Vlaanderen (AGIV). For *O. ostertagia*, the mean level of antibodies per municipality was presented. For *F. hepatica*, the percentage of farms with high level of exposure (> 0.8 ODR for the UGent ELISA and > 80 S/P for the Fasciolosis Verification Test) was mapped. Finally, the number of sampled herds per municipality was mapped.

In order to estimate the potential costs associated with the production losses induced by helminth infections, the ELISA results collected in Flanders were used in the ParaCalc® spreadsheet model (Charlier et al., 2012). This model estimates the annual cost induced by GI nematode and *F. hepatica* infection based on the observed associations between diagnostic test results and animal performance as well as the cost of anthelmintic treatment. Here, only the costs in the adult dairy herds that were related to production losses were considered, because no information was collected on the infection status of the young animals or on anthelmintic treatments.

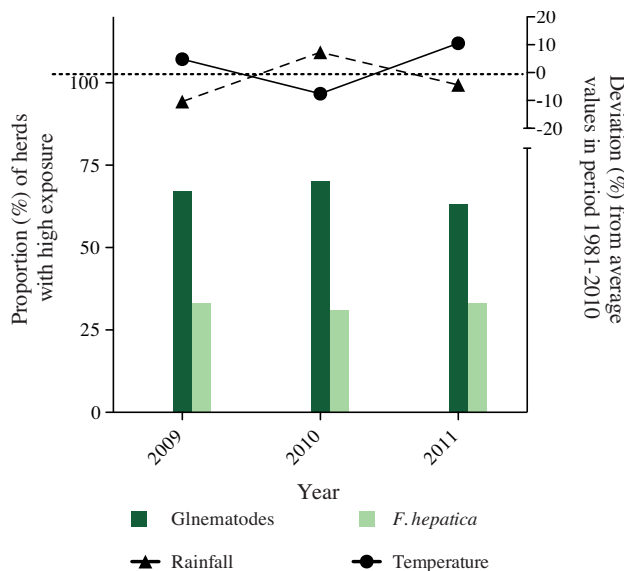
## RESULTS

### Sampled farms

The number of participating farms increased with circa 40% from 2009 until 2011 (Table 1). The number of sampled farms in 2011 corresponded to 16% of the total population of herds of dairy cows in Belgium



**Figure 1.** The number of herds sampled per municipality in 2011.



**Figure 2.** Proportion of herds with high levels of exposure to gastrointestinal nematodes and *F. hepatica* (plotted on left Y-axis). On the right Y-axis, the % deviation from normal values of yearly rainfall and mean annual temperature as recorded by the Belgian RMI in Uccle are plotted for the three years of the campaign.

(N = 10767) (Statbel, 2012). The number of sampled farms per municipality is shown in Figure 1. In general, the regions with the highest density of dairy herds (the provinces of West-Flanders, East-Flanders and the northern parts of the provinces of Antwerp and Limburg (GLOWORM report on dairy cattle distribution in Europe by Avia-GIS, 2012)) were also the regions where most of the samples were taken. However, in other regions with a high dairy herd density such as, the province of Hainaut and (the western part of) the province of Liège, many municipalities remained un- or lowly sampled.

**Table 1.** The number of participating herds and veterinary practices in the bulk-tank milk monitoring campaign for helminth infections in Belgium from 2009 until 2011.

Region	2009		2010		2011	
	Vets	Farms	Vets	Farms	Vets	Farms
Flanders	138	805	159	940	173	1116
Wallonia	85	411	112	627	128	615
Total	223	1216	271	1567	301	1731

**General levels of exposure to helminth infections**

The proportion of herds with a high level of exposure to GI nematodes and *F. hepatica* is plotted in Figure 2 for the three years. The proportion of herds with high exposure to GI nematodes varied significantly between years (P = 0.001) from 67% in 2009 over 70% in 2010 to 63% in 2011. The median (25th-75th percentile) *O. ostertagi* ODR for Flanders and Wallonia were 0.85 (0.70-0.98) and 1.00 (0.87-1.12) in 2009, 0.86 (0.68-1.01) and 0.99 (0.86-1.11) in 2010 and 0.81 (0.62-0.95) and 1.02 (0.86-1.15) in 2011. The proportion of herds with high exposure to *F. hepatica* did not significantly vary between years, and remained stable around 30%. However, when the results were split per region, there was a significant drop in the proportion of infected herds from 40% in 2009 to 31% in 2011 in Wallonia (P = 0.01), whereas no such decrease was observed in Flanders.

**Geographical distribution**

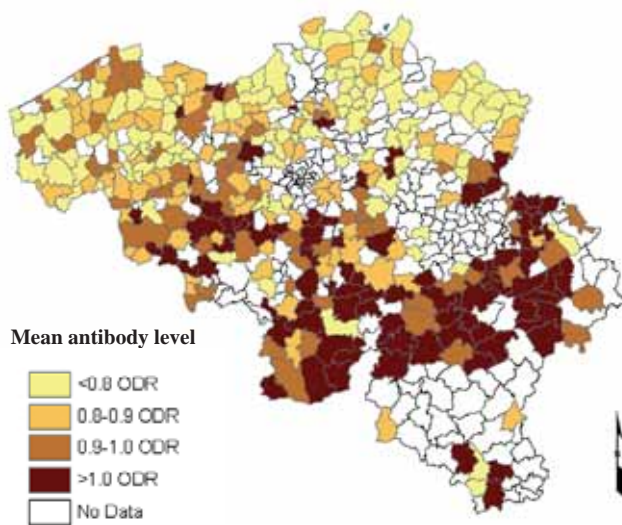
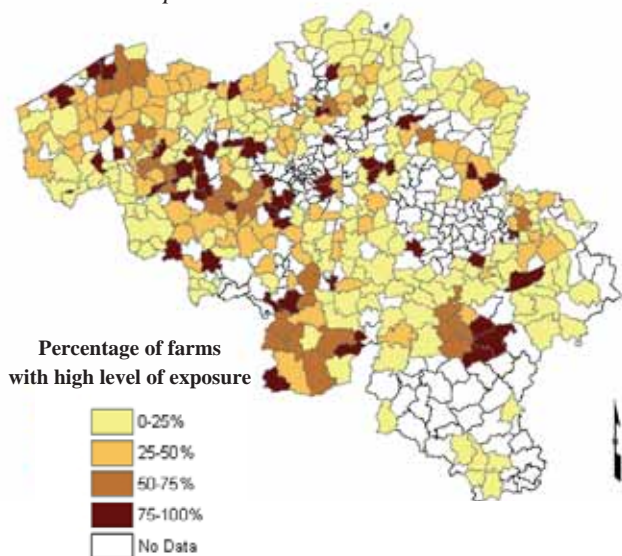
The results from the monitoring campaign of 2011 per municipality are plotted in Figure 3. It can be seen that the levels of exposure to GI nematodes increased from the north to the south of the country with the highest levels of exposure in the provinces of Namur and Liège (Figure 3a). The distribution of *F. hepatica* was more focal with infections being concentrated in the provinces of West-Flanders, the southern part of East-Flanders, Hainaut and the northern part of Luxembourg (Figure 3b). Over the three years, the average proportion of herds with high levels of exposure to GI nematodes was 58% and 83% in Flanders and Wallonia, respectively. The average proportion of herds with high levels of exposure to *F. hepatica* in that period was 31% and 34% in Flanders and Wallonia, respectively.

**Estimated costs of parasitic infections**

The median (25th - 75th percentile) (minimum-maximum) estimated annual cost caused by production losses due to GI nematode infection in Flemish dairy herds was € 45 per cow (€ 18 - € 64) (€ 0 - € 237). The median (25th - 75th percentile) (minimum-maximum) estimated annual cost caused by production losses due to *F. hepatica* infection in Flemish dairy herds was € 5 per cow (€ 0 - € 39) (€ 0 - € 101).



## A. Gastrointestinal nematodes

B. *Fasciola hepatica*

**Figure 3.** The geographical distribution of bulk tank milk ELISA results from the monitoring campaign in 2011: (A) level of exposure to gastrointestinal nematodes; (B) level of exposure to *F. Hepatica*.

## DISCUSSION

Since its introduction, the monitoring campaign for helminth infections in Belgium has an increasing participation rate. This highlights the recognized importance of GI nematode and liver fluke infections in Belgium, and suggests that the monitoring campaign is generally found to make a positive contribution to parasite control.

The large number of sampled herds allow to make some general inferences, although the voluntary aspect of the campaign should be considered, meaning that farms with an interest in or with previous parasite-related problems may be over-represented in the sample. For the comparison of results between years, it should also be considered that the sample does

not represent the same cohort of farms that was followed-up in time, but represents a different sample each year.

When the results of this campaign are compared with the results of recent surveys in surrounding countries, it can be concluded that the levels of exposure to *F. hepatica* are lower than the reported values in the UK, Northern Germany and Austria (Matt et al., 2007; McCann et al., 2010; Kuerpick et al., 2012). However, the exposure to GI nematodes is higher than reported elsewhere in Europe (Forbes et al., 2008; Bennema et al., 2010).

Within Belgium, there were also important regional differences. The distribution map (Figure 3) shows a high level of agreement with the previously reported spatial distribution of GI nematode and *F. hepatica* exposures in Flanders (Bennema et al., 2009; 2011), and provides to the authors' knowledge the first (albeit incomplete) data on the geographical distribution in Wallonia since 1997 (Lonneux et al., 2000). A remarkable observation is the large difference in exposure to GI nematode infection in Flanders compared to Wallonia. Differences in grazing management (e.g. longer pasture season, higher proportion of grazed grass in cow's diet) are believed to be the main driver of this difference, although precise data are lacking to ground this hypothesis.

The estimated annual costs of the infection indicate that the losses caused by both infections on Belgian dairy herds should not be neglected. The results suggest a wide variation in the helminth-associated costs between farms, indicating the need to tailor control programmes to the situation on individual farms. The presented data may be used to identify realistic economic target values for individual farms in order to find an equilibrium between the costs of control efforts and the costs of suffered production losses. The web-accessible ParaCalc module ([www.paracalc.com](http://www.paracalc.com)) developed by Ghent University may help in this respect (Charlier et al., 2012).

Finally, the following question should be considered: "Does monitoring helminth infections by bulk tank milk ELISA's help to make progress in parasite control?" The results suggest that despite 3-4 years of monitoring, the levels of exposure have remained relatively stable over the last three years, and only small decreases in exposure were observed. Three factors could explain this observation: 1) the step from monitoring to installing a control programme, 2) the currently applied control approaches and 3) possible counter-acting drivers.

Monitoring helminth infections is only a first step towards effective parasite control. It is followed by many other steps before a control programme can be installed or changed: the evaluation of helminth exposure of young stock, the analysis of the grazing management, the analysis of anthelmintic treatment strategies, economic and social factors that influence the decision of the farmer (Ellis-Iversen et al., 2010). Bulk milk ELISA results can only effectively support

anthelmintic control decisions if they are considered together with these other parameters.

Previous data collections indicated that the two main anthelmintic treatment approaches in dairy cows in Belgium are whole-herd treatments during summer (against clinical dictyocaulosis) or in autumn and winter (against GI nematodes). Due to the lack of anthelmintics against *F. hepatica* with zero withdrawal time for milk, often only a part of the herd is treated during winter. Treatments applied during autumn or winter are useful to reduce detrimental effects of the infection on production (Genicot et al., 1991; Charlier et al., 2007), but are unlikely to result in large decreases of levels of exposure because pasture infection levels will be poorly affected. When control measures are targeted at minimizing levels of exposure rather than inducing production responses, a larger impact may be expected from prophylactic treatment protocols and interventions through pasture management. However, the effect of these approaches on productivity in adult cattle has poorly been evaluated to date (Gibb et al., 2005; Mason et al., 2012).

Finally, several authors have warned that in the future, the level of infection with helminths, may increase. Both the incidence of fasciolosis and of parasitic gastro-enteritis has been reported to increase in some EU member states. This trend has been primarily attributed to climatic changes, altering the survival of free-living or intra-molluscan stages on pasture (McCann et al., 2010; van Dijk et al., 2008; 2010; Fox et al., 2011; Fairweather, 2011). During the three-year period of monitoring, there were considerably large differences in rainfall and temperature (2009 and 2011 were considered “exceptionally warm” years by the RMI, whereas 2010 was considered “exceptionally cold”) (Figure 2). However, this appeared not to have a measurable impact on the level of exposure to *F. hepatica*. The increased exposure to GI nematodes in 2010 could perhaps be related to the higher rainfall in that year. However, to date, the effect of climatic changes on epidemiology of GI nematodes has received little attention, and there is a need for a long-term monitoring programme and the development of life-cycle based transmission models in order to better understand possible climate-driven alterations in infection risks (Fox et al., 2012).

In conclusion, the results are reported from a voluntary monitoring campaign on the level of exposure to GI nematodes and liver fluke in Belgium between 2009 and 2011. The levels of exposure are generally high but there are important regional variations. The exposure to GI nematodes seems considerably higher in the southern part of the country, whereas *F. hepatica* infections are concentrated in certain foci. During the three years of monitoring, only small decreases in levels of exposure were observed. The aim of monitoring helminth infections in dairy cattle is to assist in detecting and acting against parasite-induced produc-

tion losses. This is not equivalent to the reduction of the measured levels of exposure per se. However, if the aim is to maintain low levels of exposure throughout the year, there is a need to evaluate the effects of new control methods based on altered timings of treatment and changes in pasture management.

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## Uit het verleden

### KATTENSCHURFT

“Dezelve randt den kop aen, van waer de ziekte zich langzamerhand over het gansche lichaem tot aen het einde der lidmaten verspreidt.

*Verschyngselen.* - De ziekte vangt aen met eenen roosachtigen uitslag; de huid springt open, de haren plakken bij elkander en vormen met een uitlopend vocht, eene harde korst. De oogleden zyn verdikt, de oogen byna gesloten, de eetlust gaet verloren, het dier vermagert, en als men de ziekte haren vryen loop laet, dan maekt zy menigvuldige slagtoffers. Het kattenschurft is zyn ontstaen verschuldigt aen de schurftmyt.

*Behandeling.* - Eene ligte oplossing van creosote doodt de myt en herstelt zeer spoedig het dier.

*Maet-regelen van gezondheids-policee.* - De gevallen, waerby de kattenschurft op den mensch wordt overgebracht, zyn menigvuldig genoeg om de aenbeveling der noodige voorzorgen tegen deze kwael niet overbodig te maken.”

Uit:

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