SVANOVIR® *O. ostertagi*-Ab
The new standard for gastrointestinal nematode diagnosis in dairy cattle
Summary

Gastrointestinal nematode infections are a common and important cause of decreased animal health, welfare and productivity in pasture-based cattle, including dairy. The control of these parasitic infections is critical to produce “more with less”, i.e. maintaining animal productivity while improving animal welfare and reducing the frequency of unnecessary anthelmintic treatment.

SVANOVIR® *O. ostertagi*-Ab ELISA is a pioneer tool enabling the assessment of severity of infection caused by gastrointestinal nematodes and it identifies herds that have potential to enhance milk production through helminth control practices in dairy cattle.

This diagnostic technical bulletin describes the impact of gastrointestinal nematodes on production and the role of the ELISA in nematode management.
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1. Introduction

“Diagnosis before treatment” for sustainable livestock farming

The competitiveness of livestock businesses is under pressure because of increasing competition for land and raw materials and increased societal expectations on sustainable production methods. To produce “more with less” while improving animal welfare standards and the environmental impact of livestock production is one of the key challenges in agriculture for the coming decades. Infectious disease control in animals is fundamental to reach these targets. In particular, endemic diseases deserve more attention because they often remain under the radar and they potentially have a major impact on the productivity and ecological footprint of today’s farms.

**Gastrointestinal nematode infections** are a common and important cause of decreased animal health, welfare and productivity in pasture-based cattle, including dairy. In adult cattle, the single most important nematode species is *Ostertagia ostertagi* (*O. ostertagi*).

All pastured herds are at risk of exposure to *O. ostertagi* while severity of infection can vary. A European survey found significant levels of exposure with related parasite-induced production losses likely being present in 10-50% of the herds, depending on the region. The control of these infections has traditionally relied on strategic “calendar-based” treatment of cattle with anthelmintics. However, with indiscriminate and intensive use of these drugs comes a more rapid development and subsequent spread of nematode populations that are resistant to the applied anthelmintics.

**The industry is now embracing more sustainable control methods,** where strategic or repeated blank treatments are replaced by approaches that are more targeted and that employ “diagnosis before treatment”. This ensures that the anthelmintics are used where they are needed most and minimises the development of anthelmintic resistance so that they remain effective for longer.

In this bulletin, we describe why the SVANOVIR® *O. ostertagi*-Ab ELISA is today an important tool for establishing sustainable gastrointestinal nematode practices in dairy cattle.

**Impact of gastrointestinal nematodes in grazing cattle**

All grazing cattle are exposed to a variety of helminth parasites, including gastrointestinal nematodes, liver fluke and lungworms. Of these, gastrointestinal nematodes are typically responsible for bigger economic losses because they are more prevalent, acting as “silent thieves” interfering with the efficient absorption of nutrients from the gastrointestinal tract. These parasites have been shown to impact negatively on feed intake and conversion, growth rate, carcass weight, carcass composition, reproduction efficiency and milk yield.

It is well known that the production effects of *Ostertagia* are very heterogeneous between individual animals and between different herds. A diagnostic test together with expert evaluation of grazing management is required to identify the herds experiencing significant production losses. Diagnostic test results need to be seen in context of various production parameters to allow accurate conclusions for parasite management.
1. Introduction

In first-season grazing cattle, the serum (or plasma) pepsinogen concentration is used to discriminate between different levels of Ostertagia-infection and thereby to assist in the evaluation of the likely level of interference of growth and development occurring in the tested animals. Animals that are excessively exposed show growth retardation of 30–50 kg after their first grazing season and produce less milk in their subsequent productive life. Worm egg counts in faeces (faecal egg counts, FEC), are also used for diagnosis, but these are often poorly correlated to worm burden and growth or performance.

In adult dairy cattle, the level of anti-Ostertagia IgG antibodies in milk as determined by ELISA is routinely used to evaluate the level of Ostertagia exposure occurring and whether these exposure levels are potentially interfering with productivity. Consistent negative relationships have been demonstrated between antibody levels against Ostertagia and the herd-average milk production. Some studies have also shown negative relationships with carcass weight and reproduction indices. Although important between-herd variation occurs, milk yield responses of ~1 kg per cow per day have been recorded after anthelmintic treatment in appropriately exposed herds. These gains have been shown in pasture-based dairy herds in the temperate climate areas in Europe and in Canada. The recorded responses in dairy herds under high grazing regimes (e.g. New Zealand and Australia) are in the magnitude of ~0.29–0.35 kg per cow per day. Yearly bulk tank milk testing against Ostertagia has been shown to be an effective way for farmers and their advisors to evaluate the importance of gastrointestinal nematodes in their herd and to use potential production losses in their decision-making for parasite control.
2. Current standards for sustainable worm control

Anthelmintic resistance, traditionally known as a problem in sheep farming, has now also been reported in cattle in all major cattle producing regions around the globe. It reaches prevalences > 25% in some regions. The principal species showing resistance in cattle is the less pathogenic *Cooperia* spp., but several cases of anthelmintic resistant *Ostertagia* have been reported\(^\text{12}\).

In contrast to sheep, where anthelmintic resistance is widespread, anthelmintic resistance in cattle is considered to be still emerging. Scientists believe that if sustainable treatment methods are implemented now, the efficacy of current anthelmintics can be preserved, securing the effective control and economic benefit of worm control measures for the foreseeable future.

Two important new concepts have emerged concerning the sustainable use of anthelmintics: (1) **targeted treatments (TT)**, where the whole flock/herd is treated based on an evaluation of the current herd risk that uses both epidemiological knowledge and diagnostic parameters that appropriately quantify the severity of infection, and (2) **targeted selective treatments (TST)**, where only individual animals within a grazing group are treated, based on appropriate single or multiple treatment indicators (e.g. weight gain)\(^\text{13}\). The aim of Targeted and Targeted Selective Treatments are to enable effective control of nematode-induced production impacts whilst also preserving anthelmintic efficacy through the maintenance of an adequate pool of untreated parasites within hosts and on pasture that can pass on their genes to the next generation. In combination with information on pasture and grazing management (i.e. general nematode risk factors), the SVANOVIR\(^\circledR\) *O. ostertagi*-Ab ELISA applied on bulk tank milk is the primary parameter to ensure the targeting of anthelmintic treatments is towards dairy herds with the highest worm exposure. Moreover, a recent study indicated that when the ELISA on bulk tank milk is combined with cow level grazing management and treatment information, highly specific treatment recommendations can be made at the level of the individual animal\(^\text{14}\).

**Diagnostic options**

The primary indicators used to guide anthelmintic treatment decisions in **first-season grazing cattle** are:

- clinical aspects (e.g. hair coat, body condition);
- grazing management information (e.g. time of turnout on pasture, length grazing season);
- laboratory tests (FEC and the serum/plasma pepsinogen concentration)\(^\text{15}\).

The former 2 are easy to perform but either detect nematode problems too late (after impact has already occurred) or are not definitive enough in their own right (grazing management). The laboratory tests are more specific indicators of infected animals but require animal handling, involve a delay whilst awaiting laboratory results and are more expensive.

In temperate climate regions, where animals are housed over winter, FEC are mainly useful to evaluate nematode challenge and guide herd-level anthelmintic treatment decisions in the middle of the grazing
2. Current standards for sustainable worm control

season once infection levels are established in animals. Pepsinogen is mainly used at the end of the grazing season, or later when the animals are stabled, to evaluate the level of Ostertagia present at housing and the exposure to Ostertagia that has occurred over the past grazing season so as to adapt the worming regime in the next season. FEC and pepsinogen are useful to guide herd level anthelmintic control measures but are not adept for selecting out the individual animals requiring treatment. Arguably the best parameter for this purpose is productivity measures such as weight gain which can be monitored at regular intervals over the grazing season. Yet, in the absence of automated animal handling and weighing facilities on farm, this monitoring and treatment approach remains impractical in most current circumstances.

In dairy cows, the SVANOVIR® O. ostertagi-Ab ELISA detecting antibodies against gastrointestinal nematodes in bulk tank milk can be combined with evaluation of the grazing and anthelmintic treatment history of the animals as the primary parameters to use for monitoring exposure to gastrointestinal nematodes and the potential for production losses. However, more recently additional parameters such as the time of effective contact (TEC) with pasture and farmer technicity have both been proposed as information that can assist in fine-tuning the interpretation of ELISA results. Other parameters such as FEC, pepsinogen and age have been evaluated to be included within anthelmintic treatment decision approaches during the risk period but have been found unsuitable as they did not reflect appropriately the herd’s parasite exposure (level or risk), nor were they consistently linked to herd productivity changes after anthelmintic control measures.

Comparison of current diagnostic options for gastrointestinal nematode infection in dairy cattle

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>±</td>
</tr>
<tr>
<td>Usable for first-season grazing calves</td>
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<td>√</td>
<td>√</td>
<td>-</td>
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<tr>
<td>Usable for adult cattle</td>
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<td>-</td>
<td>-</td>
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</table>

* The use of Svanovir® O. ostertagi ELISA for application in first-season grazing calves is currently being evaluated (See section "Recent scientific results").
2. Current standards for sustainable worm control

Practical guidelines for sampling and interpretation

The SVANOVIR® *O. ostertagi*-Ab ELISA is a semi-quantitative test, measuring the level of IgG antibodies the cows have generated after being exposed to gastrointestinal nematodes whilst they are grazing on pasture. The test results are expressed as optical density ratios (ODR), with a high ODR indicating a high exposure to gastrointestinal nematodes or at least that the exposed cattle are reacting significantly to the level of challenge present. Both are likely to be interfering with animal productivity. Combining the ODR result with further assessment of the farm goals, farm type, grazing and treatment history is the current way to optimize the employed anthelmintic control strategy and make sustainable anthelmintic treatment decisions 15.

The test is not suitable for evaluation of the efficacy of an anthelmintic treatment because the antibodies remain present for months after treatment. It takes also about 6 weeks time for the antibodies to build up following recent parasite exposure and to see the effect in the ELISA result. The recommended approach is to either take a yearly sample towards the end of the grazing season or after periods of suspected maximal exposure risk. This allows to evaluate the exposure of the cows to the parasites and potential production losses in a particular year as a starting point to adjust the anthelmintic control strategy.

In herds with seasonal calving, the test can be aligned with the calving pattern so that the diagnostic information can support the optimal commencement of lactation. When the testing is repeated in the same month over several years, it allows to evaluate longer term trends in parasite exposure as a result of applied control measures as well as meteorological conditions 19.
2. Current standards for sustainable worm control

*Figure 1* shows an interpretation chart of ODR values and how these values are linked to potential milk production losses. This relationship is based on a study conducted in Belgium\(^{20}\), and has been confirmed in studies in Canada\(^{21}\), France\(^{22}\) and Spain\(^{23}\). In general, ODR values > 0.8 reflect a high nematode exposure that is likely to be causing significant milk yield losses. However, the interpretation can vary with differences in local epidemiological and farming conditions.

As an example how to read the plot, we have highlighted a hypothetical ELISA result of 1.0 ODR in red. On the left bar, you can read that 16% of the herds in your country/region have the same result and that you are positioned in the higher quarter of observed nematode exposure levels. On the right bar (by following the red arrow), you can read that this level of exposure corresponds to an expected milk yield loss of about 1.5 kg/cow per day.

However, there is no guarantee that milk yield will increase after anthelmintic control measures based on bulk milk analysis in the individual herd. The results should be assessed with reference to the holistic herd health situation and production status.

*Figure 1. Interpretation chart for SVANOVIR® O. ostertagi-Ab ELISA with example as explained in the text. Bars indicate observed frequency distribution in a certain region/population. The line plots the potential milk yield loss associated with a certain test result.*
3. SVANOVIR® *O. ostertagi*-Ab field applications

Introduced in the market in 2008, the SVANOVIR® *O. ostertagi*-Ab ELISA has subsequently been used incrementally in several countries around the world. In many of these countries the application of the test has been evaluated and adapted to fit the local conditions and farming methods. Below we give a number of examples, with particular focus on Europe.

“Porte d’entrée” for herd health management on parasitic infections in France

In France, the test is routinely used at the end of the grazing season (autumn). A fixed annual sampling date is recommended to allow optimal comparison of the results from previous years. The result is included in the annual health surveillance report, performed by the associated veterinarian. The test is regarded as a port of entry to discuss and optimize the management of gastrointestinal nematode and other parasitic infections in a herd. Two technical committee meetings have been held, hosted to date by Merial, to discuss optimal sampling regimes and interpretation. A threshold of 0.7 ODR is applied. Test results above this threshold call for further investigation and potential adaptation of the nematode management on a farm.

Worm bulk tank milk programme in the Netherlands

In the Netherlands, farmers can subscribe to the bulk tank milk monitoring programme provided by GD Animal Health. Besides gastrointestinal nematodes, farmers can also subscribe to monitor for antibodies against liver fluke or lungworm infections. The programme is used to check which parasites are present on the farm and to what extent. About 2000 farmers participate in the programme and samples are collected each year in October/November (autumn). The results are communicated to vets and farmers to enable optimization of farm worm control programmes for the subsequent season, with a focus on the prudent use of anthelmintics.

Parasitic Profile in Belgium

In Belgium, over 1200 dairy farmers participate annually in a programme that measures antibodies against gastrointestinal nematodes and liver fluke in bulk tank milk. The tests are carried out in autumn (October/November) and are used to evaluate the exposure to both parasites and the potential production losses. Farmers are encouraged to also participate in pepsinogen testing to evaluate the nematode pressure in the young stock. The combined results are used by the veterinarian to improve the grazing management and anthelmintic treatment practices on farm. In addition, the results can be fed into a web-application (ParaCalc.com) to estimate the potential financial losses associated with the worm burden on a farm. The monitoring programme was developed by DGZ Vlaanderen, Ghent University and Merial.
3. SVANOVIR® *O. ostertagi*-Ab field applications

**Monitoring programme outside of Europe**

In Oceania, dairies are often run as seasonal calving operations with grazing occurring all year round. Worm challenge does vary with season but the combination of high stocking rates and irrigated pastures can ensure the presence of nematodes all year round. The constancy of challenge and the concentration of cows in early lactation in spring ensure that the focus of testing is on whether or not a treatment in early lactation is required. Samples taken in spring or towards the end of the previous lactation cycle (autumn) provide key data for the decision-making process.
4. Frequently asked questions

What can the test do?
The SVANOVIR® *O. ostertagi*-Ab ELISA is a semi-quantitative test, measuring the level of IgG antibodies the cows have generated after being exposed to gastrointestinal nematodes whilst they are grazing on pasture. The test results are expressed as optical density ratios (ODR), with a high ODR indicating a high exposure to gastrointestinal nematodes or at least that the exposed cattle are reacting significantly to the level of challenge present. Both are likely to be interfering with animal productivity. Combining the ODR result with further assessment of the farm goals, farm type, grazing and treatment history is the current way to optimize the employed anthelmintic control strategy and make sustainable anthelmintic treatment decisions.

When is the best time to sample?
In order to evaluate the exposure of the cows to the parasites and potential production losses in a particular year as a starting point to adjust the anthelmintic control strategy, we recommend to either take a yearly sample towards the end of the grazing season or after periods of suspected maximal exposure risk.

In herds with seasonal calving, the test can be aligned with the calving pattern so that the diagnostic information supports decision making in the strategic control of Ostertagia. When the testing is repeated in the same month over several years, it allows to evaluate longer term trends in parasite exposure as a result of applied control measures as well as meteorological conditions.

When should I repeat the test to evaluate efficacy of treatment?
The test is not suitable for evaluation of the efficacy of an anthelmintic treatment because the antibodies remain present for months after treatment. In the absence of re-infection, it takes about 4 months for antibodies to drop after anthelmintic treatment. In case animals remain on pasture and are exposed to re-infection, no to a minimal drop in antibodies may be observed. The only available test to evaluate the efficacy of anthelmintic treatment is the faecal egg count reduction test, based on worm egg counts in faeces before and 7–14 days after anthelmintic treatment. Further, it is important to evaluate the effect on animal health and (milk) production after anthelmintic treatment.

Is bulk tank milk ELISA also a suitable diagnostic measure in big dairy herds?
Most of the evaluation work of the *O. ostertagi* ELISA has been performed on herds with a size of 50–300 dairy cows. Within these herd sizes, good correlations have been observed between test results obtained from the bulk tank milk and the mean of the individual cow milk samples. The *O. ostertagi* ELISA has also been applied on bulk tank milk from bigger herds, with apparent good representation of the average exposure level. However, it has been shown that within herds, with either a low or high bulk-tank milk *O. ostertagi* ELISA result, there is always a big variation in the individual cows’ ELISA results. Therefore, by using a bulk-tank milk sample only the
average nematode exposure of a herd is estimated, while important variation is present at the individual level. In addition, the test results of bulk-tank milk samples taken only a few weeks apart can show considerable variation depending on calving pattern, the number of cows contributing to the tank and their relative milk yields and antibody titers. The average of individual milk samples will be less influenced by these factors and also assesses the within-herd variability.

**Can individual milk samples be used for the test?**
When no bulk tank milk samples are available, the test can be performed on individual milk samples. As mentioned above, the average of individual milk samples is less subject to variations in test results caused by changes factors such as cows contributing to the bulk tank herd composition, relative milk yield of cows contributing to the bulk tank. The best results are obtained when all lactating animals are sampled. However, field data suggest that the sampling of 10 samples from first lactation cows and 10–15 from higher lactation cows can be used to investigate if there are differences in nematode infection pressure of pastures grazed by the heifers vs. adult cows. In order to interpret the data, it should be considered that the average ELISA result of individual milk samples is always lower than the bulk tank ELISA result by approximately 40% (Conversion formula: Individual ODR = 0.09 * 0.50 * bulk tank milk ODR) \(^{26}\). The *O. ostertagi* ELISA is at current not considered as a suitable measure to identify individual animals requiring treatment.

**Can serum be used for the test?**
The SVANOVIR® *O. ostertagi*-Ab ELISA has been used in several epidemiological studies on cow’s (mainly beef calves/cows) plasma or serum to measure differences in nematode exposure according to different management practices, weather patterns or geographic locations \(^{27-29}\). The test remains identical, but a dilution rate of 1/140 is applied to the sera. A drawback is that no interpretation standards have been provided so far. The most promising use is on calves’ sera, where it could lead to more cost-effective diagnosis compared to the pepsinogen assay. Good correlations with the pepsinogen test have been shown and a threshold of 0.7 ODR has been proposed to identify first-season grazing cattle suffering growth retardation caused by gastrointestinal nematodes \(^{30}\). Further research is required to confirm these findings.

**How specific is the test?**
The SVANOVIR® *O. ostertagi*-Ab ELISA detects antibodies directed to adult worm extracts of *O. ostertagi*. Cross-reactions with other gastrointestinal nematodes such as *Cooperia* and with liver fluke and lungworms may occur occasionally \(^{35}\). At the time very little is known about the incidence of co-infections and further investigations are ongoing.
4. Frequently asked questions

How reproducible is the test?
The SVANOVIR® *O. ostertagi*-Ab ELISA is known for its good reproducibility within and between labs. This is an important advantage of the test compared to e.g. the pepsinogen assay. When the same samples were repeatedly tested in 4 different laboratories, 1% of the variation was attributable to the day and 2% to the laboratory. Still, variations between labs occurred, highlighting the importance of continued training, quality control and regular ring-testing. When the same sample was tested in different laboratories, deviations in ODR readings between -0.23 and 0.23 included 95% of the observations. Reproducibility can be further improved by testing samples in duplicate on the same plate.

How important is correct milk handling?
The effect of various milk handling procedures on *O. ostertagi* ELISA results have been tested. This is important as the ELISA is often applied on milk samples collected for milk quality or other dairy herd improvement programmes. These samples often undergo various handling procedures such as addition of a preservative (e.g. bronopol), heating for milk component analysis and somatic cell counting. In other cases, samples may be frozen for collection until sufficient samples have been collected for analysis. Research showed that the test is resilient to most of these stressors. For instance, an extreme treatment where milk samples were heated, frozen, thawed, and re-frozen for 4 weeks resulted in a difference less than 5% of the observed ODR range. Other studies showed that using whole milk instead of skimmed milk, the addition of bronopol, freezing up to 8 months and up to 2 extra freeze-thaw cycles of the milk samples did not significantly affect the test results. Especially the usability of whole milk instead of skimmed milk is an important finding as this can save lots of time in the lab.

On the other hand, it has been shown that *O. ostertagi* ELISA results are correlated with somatic cell counts or mastitis indicators in individual milk samples. Therefore, the use of composite milk samples, which have less variable somatic cell counts than samples taken from each quarter, are more suitable when the udder health status is unknown. Further, milk from clinically infected quarters should not be added to the bulk tank milk.
5. Conclusion

Introduced in the market in 2008, the SVANOVIR® O. ostertagi-Ab ELISA has meanwhile become an essential tool to evaluate worm exposure in dairy herds. It is used in thousands of herds in various regions around the globe and supports farmers in optimizing the health and productivity of their cattle. The general use of the test is to analyze a bulk tank milk sample to assess the level of gastrointestinal nematode exposure at housing or after periods of suspected maximal exposure risk. In herds with seasonal calving, the test can be aligned with the calving pattern so that the diagnostic information can support an optimal profitability. When the test is repeated in the same period each year, it allows to establish the effect of control advices and meteorological conditions on nematode exposure and thus to take informed anthelmintic control decisions. The test can be integrated in a larger monitoring scheme including parasitic infections of importance in young stock or adult cows (e.g. liver fluke and lung worm) to develop a more holistic advice on worm control. By supporting the targeted use of anthelmintics to those animals that are at proven risk of production losses, the test supports the industry in making the shift towards sustainable control practices and has become the current standard in gastrointestinal nematode diagnosis in dairy cattle.
I started my scientific career as a PhD student at Ghent University on a project called “The use of anti- _O. ostertagi_ antibodies in milk to study the epidemiology and impact on production of gastrointestinal nematode infections in dairy cows”. I am very proud that my research contributed to the development of a commercial _O. ostertagi_ ELISA kit in collaboration with Boehringer Ingelheim Svanova. This commercialisation allowed that the test is no longer only used in the labs of universities and research institutions, but is now available for use and further research around the globe.

The negative impact of gastrointestinal nematodes on milk production in adult cattle was a subject of intense debate at the start of my research. In the meantime, the assay has greatly contributed to show the deleterious impact of gastrointestinal nematodes on production in adult cattle and has delivered the industry a lever to gain significant returns-on-investment from improved anthelmintic control measures. This story is still not finished because for the future we need more tools to identify individual animals requiring anthelmintic treatment as well as more insights and tools to assess the effects of parasite and disease control on the farm’s economic performance.
Located in Uppsala in the central parts of Sweden Boehringer Ingelheim Svanova develops high quality diagnostics that enable detection of antibodies to viruses, bacteria, parasites and mycoplasmas in various animal species.

Our research and development team has successfully created veterinary diagnostic solutions for over 25 years that contribute to the control of infectious diseases in animals worldwide. The robust and globally applicable assays are developed from start to finish in our own laboratories or in close collaboration with research groups around the world. We follow our products every step of the way to ensure the best diagnostic tools available on the market.

Since 2000, all our products are manufactured and supplied according to the ISO 9001:2008 quality management system.

Boehringer Ingelheim Svanova is proud to have two unique ELISAs in its portfolio that show the correlation between antibody levels and the impact on milk yield and/or carcass weight caused by infections with *O. ostertagi* and *Fasciola hepatica*. These two assays are based on a new approach to testing for parasite infection, based on a semi-quantitative measurement of the exposure to parasites in relation to an economic threshold that shows when the use of an anthelmintic is justified.

### Assay overview

**SVANOVIR® O. ostertagi-Ab**

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* Samples: Max. number of samples for analysis, wells for kit controls excluded.

**SVANOVIR® F. hepatica-Ab**

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* Samples: Max. number of samples for analysis, wells for kit controls excluded.

To find out more about our products and our company please visit [www.svanova.com](http://www.svanova.com)
8. References

8. References


