svanova

ELISA assays for parasitic and tick-borne diseases

Boehringer Ingelheim Svanova

We are passionate about the health and well-being of humans and animals. Immunodiagnostics from Boehringer Ingelheim Svanova contribute to a global, adequate supply of safe and nutritious food.

Boehringer Ingelheim Svanova offers innovative and unique ELISA assays for objective, specific and cost effective diagnostics of parasitic and tick-borne disease in livestock. This enables a targeted and sustainable treatment management strategy.

SVANOVIR® *F. hepatica*-Ab

SVANOVIR® *O. ostertagi*-Ab

SVANOVIR® *A. marginale*-Ab

SVANOVIR® *B. bigemina*-Ab

SVANOVIR® *Neospora*-Ab

Contents

Boehringer Ingelheim Svanova Immunodiagnostics

New standards in the management of parasite infections

Grazing animals are constantly exposed to parasites and this potential causes economic loss due to reduced productivity in infected animals. With the implementation of a targeted parasite management system, the negative effects of parasite infections can be held at a minimum. The methods of controlling internal parasites should be customized to fit the production situation with emphasis on a reduced parasitic exposure in the environment. To reduce the development of pharmaceutical resistance animals should be adequately diagnosed as infested with a parasitic exposure that is extensive enough to justify the use of pharmaceuticals in the parasite management.

Conventional diagnostic methods all have shortcomings as the methods often depend on the presence of the parasite or the parasite eggs in the sample, *e.g.* fecal egg count, inspection of inner organs at slaughter or blood smear examinations. These methods are often expensive, time consuming, subjective and demands high expertise.

The ELISA assay is a superior immunodiagnostic tool that enables detection of antibodies to the pathogen both during and post infection in comparison with conventional methods where the presence of the pathogen is required in the sample. The ELISA provides an objective, fast, high performing and cost effective measurement of antibodies and furthermore it is suitable for both large and small scale testing. It is also possible to run tests from several different sample materials such as serum, plasma, milk and meat juice. Thus the ELISA assay is an exceptional and essential diagnostic method for the management of parasite infections.

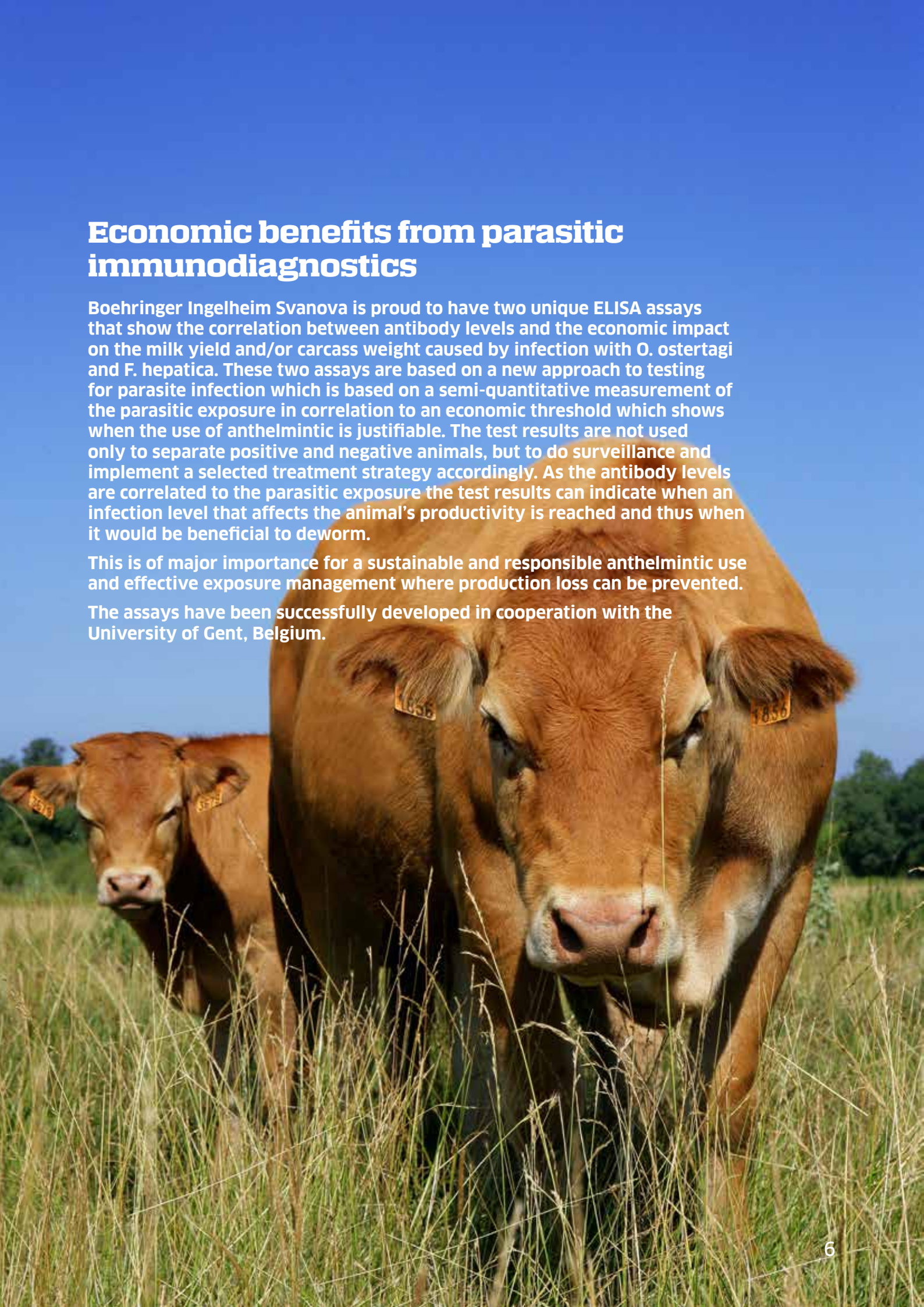
Manufactured under strict ISO 9001:2008 standards

Economic benefits from parasitic immunodiagnostics

Boehringer Ingelheim Svanova is proud to have two unique ELISA assays that show the correlation between antibody levels and the economic impact on the milk yield and/or carcass weight caused by infection with O. ostertagi and F. hepatica. These two assays are based on a new approach to testing for parasite infection which is based on a semi-quantitative measurement of the parasitic exposure in correlation to an economic threshold which shows when the use of anthelmintic is justifiable. The test results are not used only to separate positive and negative animals, but to do surveillance and implement a selected treatment strategy accordingly. As the antibody levels are correlated to the parasitic exposure the test results can indicate when an infection level that affects the animal's productivity is reached and thus when it would be beneficial to deworm.

This is of major importance for a sustainable and responsible anthelmintic use and effective exposure management where production loss can be prevented.

The assays have been successfully developed in cooperation with the University of Gent, Belgium.



SVANOVIR® *F. hepatica*-Ab for semi-quantification of the infestation level for *Fasciola hepatica* in cattle

The SVANOVIR® *F. hepatica*-Ab assay is based on an Excretory-Secretory (ES) antigen extracted from the liver fluke. This assay enables identification of herds and individuals that exceed parasitic exposures that can lead to the decrease in milk production and carcass weight.

SVANOVIR® *F. hepatica*-Ab has been validated in dairy and beef cattle using milk, serum/plasma and meat juice samples respectively, thus enabling the monitoring of fasciolosis at several different stages of the production chain, *i.e.*, at the farm, at dairies and at slaughterhouses.

PERFORMANCE CHARACTERISTICS | SVANOVIR® *F. hepatica*-Ab has been validated in comprehensive studies on naturally infected populations. In these studies, a strong correlation between the parasitic exposure (number of flukes in the liver), antibody levels to *F. hepatica*, and loss of milk yield or carcass weight was demonstrated (Charlier *et al.*, 2007; 2009). Cut-off values were determined to indicate when production loss from *F. hepatica* infestation becomes economically relevant. Furthermore, those individuals that benefit most from anthelmintic treatment could be identified as shown in Figure 1 (Charlier *et al.*, 2012).

The total increase of milk yield for the anthelmintic treated animals compared to the placebo treated animals corresponded to approximately 1kg/day per cow.

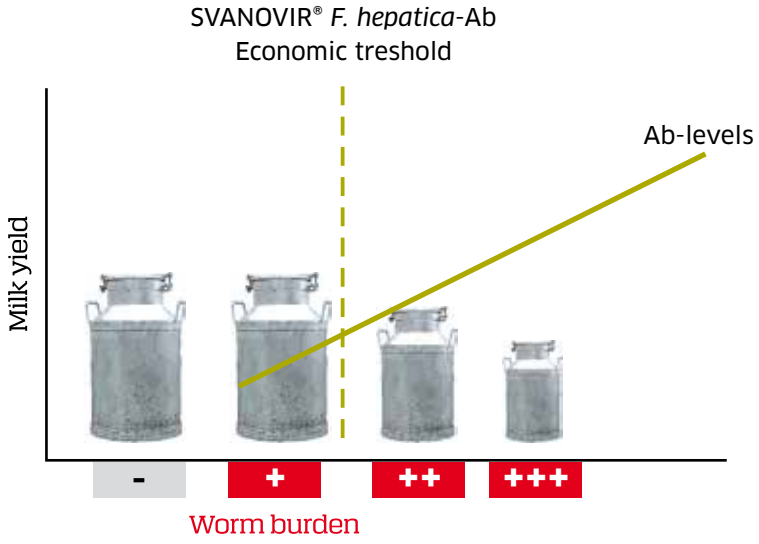
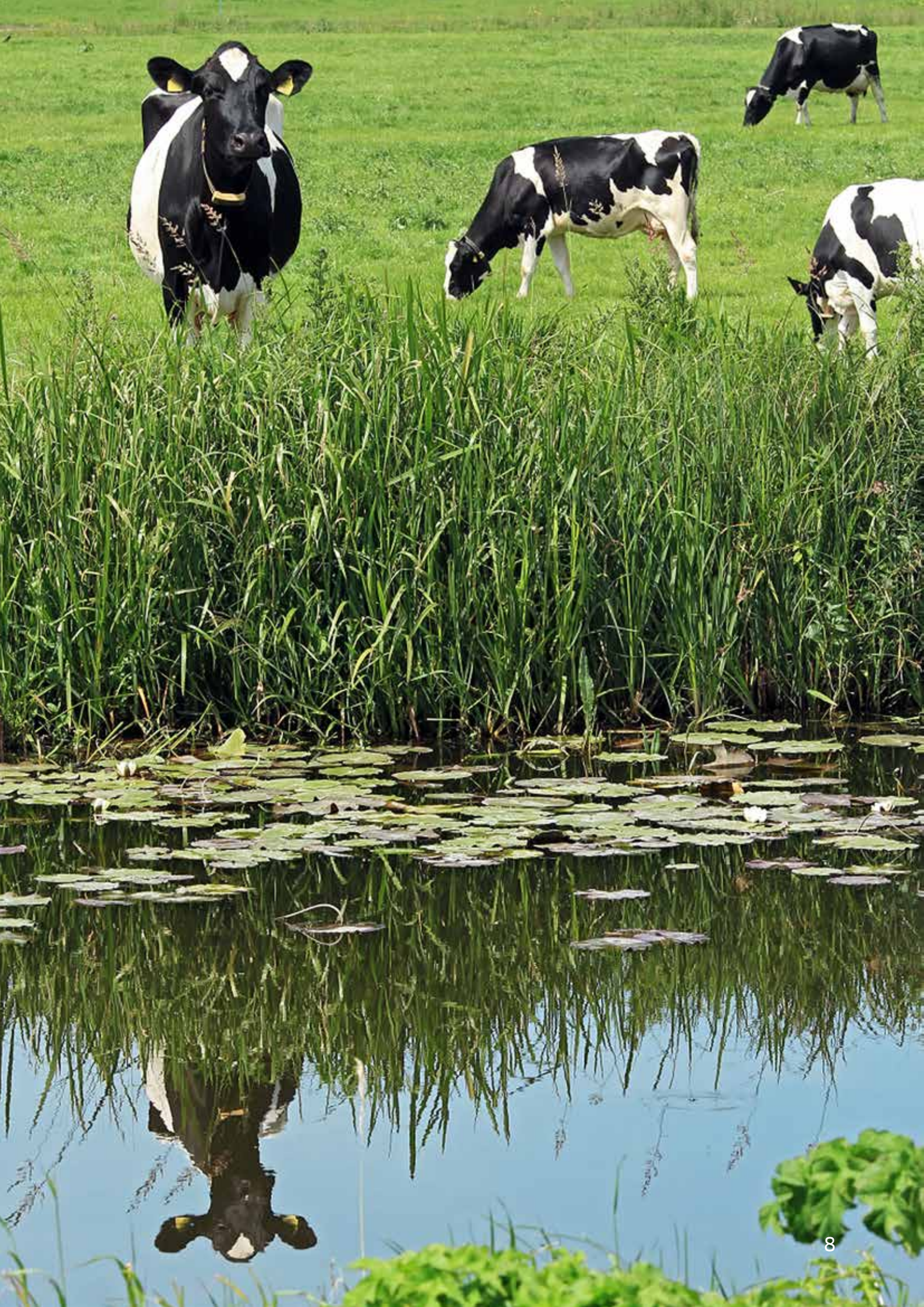


Figure 1. Cut-off values are determined to indicate when production loss from *F. hepatica* infection becomes economically relevant. Charlier *et al.*, 2012.

ASSAY OVERVIEW

SVANOVIR® *F. hepatica*-Ab			
Species	Bovine		
Samples	Serum/plasma		
	Meat juice		
	Milk, individual and bulk milk		
Type	Indirect ELISA based on E/S antigen		
Article number	**Samples***	**Plates**	**Format**
104896	184	2	Strips

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



SVANOVIR® *O. ostertagi*-Ab for semi-quantification of *Ostertagia ostertagi* in bovine

SVANOVIR® *O. ostertagi*-Ab semi-quantitative assay is the first unique ELISA for detection of antibodies to *O. ostertagi* in grazing cattle. The anti¬body levels shown in the test results are correlated to the animal's parasitic exposure and the estimated amount of reduced milk yield the infestation level can cause, as seen in figure 2.

PERFORMANCE CHARACTERISTICS | The SVANOVIR® *O. ostertagi*-Ab assay has been validated in comprehensive field studies of naturally infected cattle herds in Europe. Data from a large number of dairy farms in different European countries show a high correlation between the parasitic exposure of *O. ostertagi*, antibody levels in bulk tank milk and production loss (Charlier *et al*., 2005; Forbes et al., 2008). Therefore SVANOVIR® *O. ostertagi*-Ab is an excellent tool to evaluate the parasitic exposure in herds and assess whether anthelmintic treatment is expected to result in economic benefit or not.

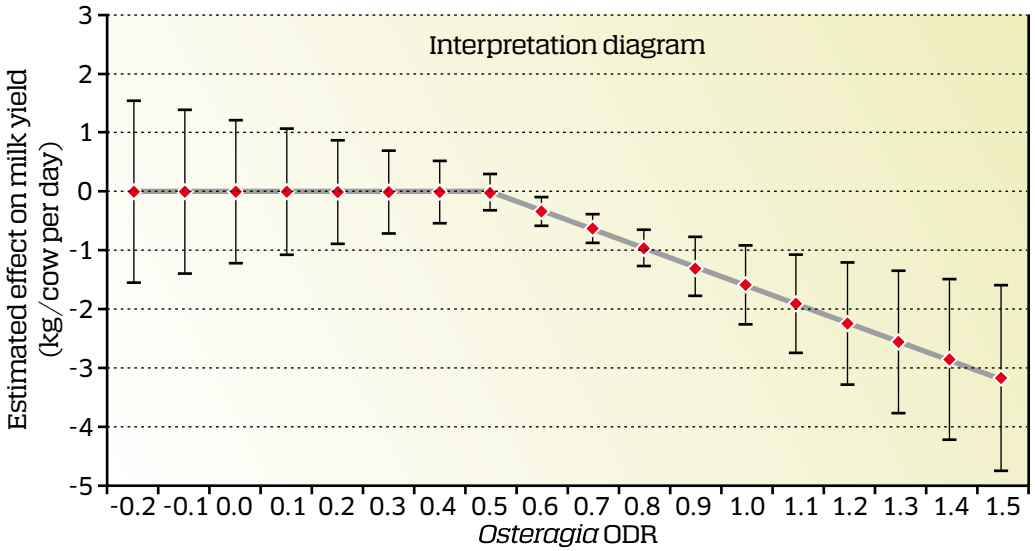


Figure 2. The relation between ODR value and change in milk yield, based on tests on bulk milk samples from >800 European herds. The deviation bars are in correlation to the number of sampled herds.

ASSAY OVERVIEW

SVANOVIR® *O. ostertagi*-Ab			
Species	Bovine		
Samples	Bulk tank milk		
Type	Indirect ELISA		
Article number	**Samples***	**Plates**	**Format**
104897	184	2	Strips

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

Tick-borne and OIE listed notifiable diseases

Worldwide farmers suffer from economic loss and significant mortality rates in livestock due to tick-borne diseases (TBD). Many of these are notifiable diseases according to the OIE manual passed by the International Committee and recommendations issued by the Regional Commissions. In order to prevent the disease from spreading a reliable test method is needed to make the implementation of an effective parasite management system feasible. Conventional test methods are not always the best method of choice due to various shortcomings such as difficult, subjective and time consuming test procedures demanding high expertise. High expenses, low sample throughput and subjective results. However ELISA assays are excellent tools in order to monitor and/or confirm a populations freedom from infection, contribute to eradication programmes, surveillance and study of infection prevalence as well as monitoring the immune status in individual animals or populations post-vaccination (whenever vaccines are available).

Our ELISA panel for TBD, *A. marginale* and *B. bigemina* are based on recombinant antigens that ensure high specificity plus excellent sensitivity. The capability of adaptation to both small and large scale testing further makes these test essential in the diagnosis of tick-borne pathogens in bovines.

SVANOVIR® *A. marginale*-Ab for detection of *Anaplasma marginale* in serum samples

SVANOVIR® *A. marginale*-Ab is based on a recombinant immunodominant antigen detecting antibodies to *A. marginale*. As an intraerythrocytic pathogen *A. marginale* is often diagnosed by subjective methods such as the Romonovski/Giemsa stained blood smears or the indirect fluorescent antibody test (IFAT), methods that are time consuming, difficult to standardise and subjective which means to obtain reliable test results, high expertise and experience is necessary. In comparison, the ELISA assay is more specific, gives fast results, is objective and cost-effective and can be applied on both large and small scale testing.

PERFORMANCE CHARACTERISTICS | SVANOVIR® *A. marginale*-Ab is an assay developed together with experts from the International Livestock Research Institute (ILRI), Nairobi, Kenya. The test is a field validated and high performing ELISA enabling the screening of cattle. The assay provides reliable identification of subclinically infected and carrier animals.
The commercialisation of the test improved its sensitivity so that experimentally infected animals could be detected already after 6 days post infection (pi.) compared to day 13 pi. with the ILRI in-house assay as seen in Table 1.

Table 1. Determination of seroconversion in *A. marginale* experimentally infected cattle from Kenya.

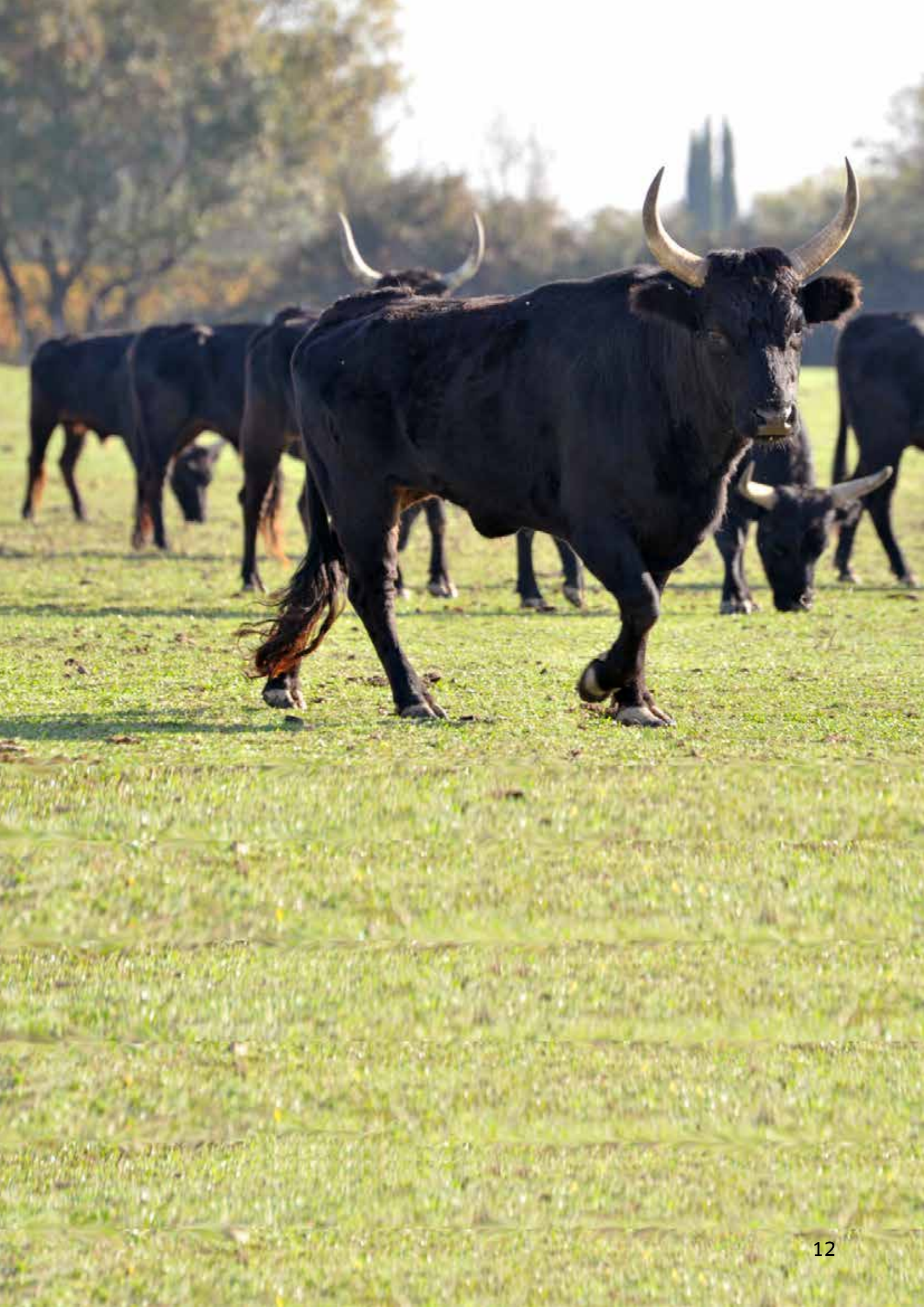
Day of experimental infection	SVANOVIR	ILRI
0	Negative	Negative
3	Negative	Negative
6	Positive	Negative
9	Positive	Negative
13	Positive	Positive
17	Positive	Positive
20 - 174	Positive	Positive
181 - 237	Positive	Positive
360	Positive	Positive

ASSAY OVERVIEW

SVANOVIR® *A. marginale*-Ab

Species	Bovine		
Samples	Serum		
Type	Indirect ELISA using a recombinant immunodominant antigen		
Article number	**Samples***	**Plates**	**Format**
104899	184	2	Strips

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



SVANOVIR® *B. bigemina*-Ab for detection of antibodies to *Babesia bigemina* in bovine

SVANOVIR® *B. bigemina*-Ab detects antibodies specific to *B. bigemina* in bovine serum samples, with high sensitivity. To find the right treatment method and prevent the disease from spreading, identifying the correct blood pathogen is of high importance. Many different diagnostic methods are available; among them immunofluorescence antibody test (IFAT) and Romonovski/Giemsa stained blood smears; but they have shortcomings such as being subjective and time consuming. The IFAT test use either whole parasites or semi-purified antigens whose qualities can vary from batch to batch making it less specific and difficult to standardise. The PCR test has a high specificity but is not suitable for large scale testing and analyses are expensive. The SVANOVIR® *B. bigemina* ELISA is easy to handle and standardise, provides objective test results with high sensitivity and specificity, and can be adapted for both large and small scale testing. It detects babeosis from day 14 pi. and antibody responses are present for a long time as seen in figure 3 (Tebele *et al*., 2000).

PERFORMANCE CHARACTERISTICS | The SVANOVIR® *B. bigemina*-Ab is based on an assay developed by the International Livestock Research Institute (ILRI), Nairobi, Kenya, with a standardised recombinant immunodominant antigen (Tebele 1996, Tebele *et al*., 2000). The ILRI ELISA sensitivity and specificity have been estimated at 96% and 97.5% respectively (Tebele, 1996). There is no cross-reactivity to *Babesia bovis*, *Theileria parva*, *Theileria taurotragi* or *Anaplasma marginale* (Morzaria *et al*., 1992). The ELISA has also been shown to have high agreement with IFAT (Tebele, 1996).

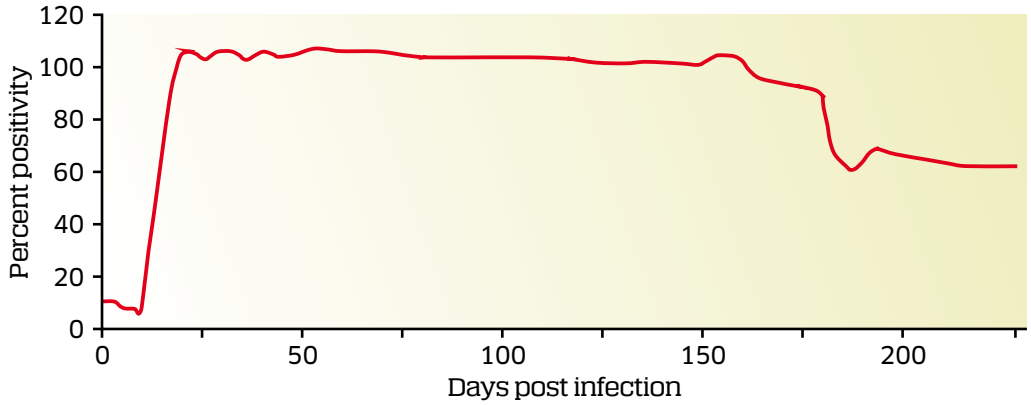


Figure 3. Antibody response from a steer experimentally infected with *B. bigemina* sporozoites. Sera was collected over a period of 228 days and a significant Ab response was developed and detectable by day 14 and maintained at a high level until day 179 after which the response fell to approximately 60% its maximal level by day 228. Tebele *et al*., 2000.

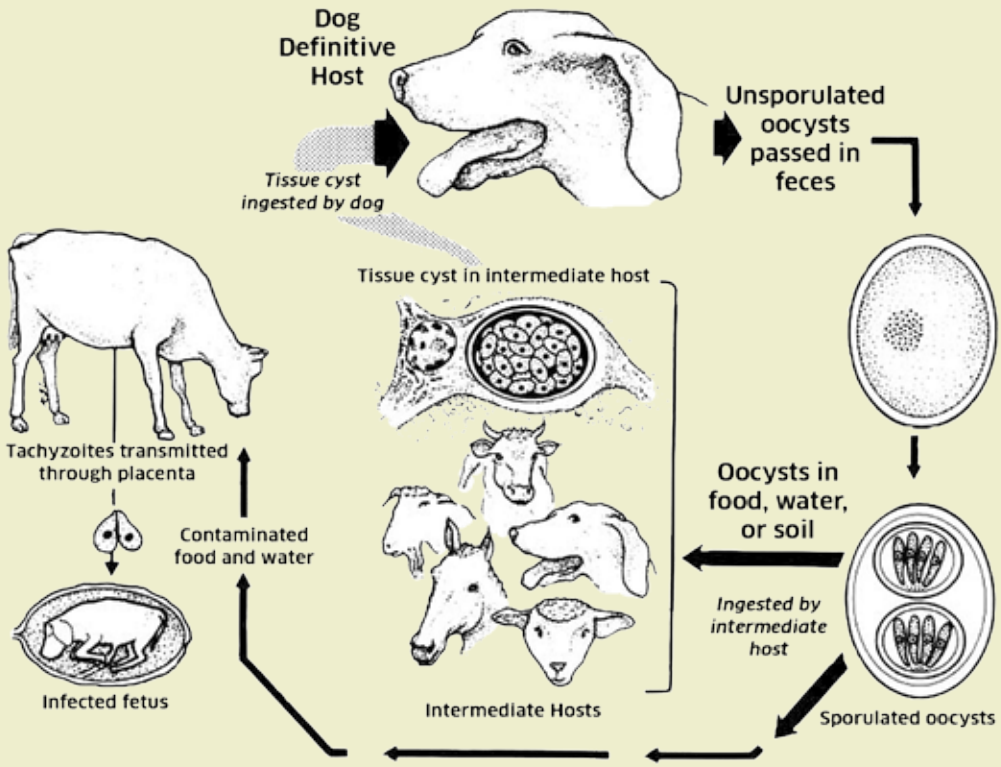
ASSAY OVERVIEW

SVANOVIR® *B. bigemina*-Ab			
Species	Bovine		
Samples	Serum		
Type	Indirect ELISA using a recombinant immunodominant antigen		
Article number	**Samples***	**Plates**	**Format**
104900	184	2	Strips

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

Detection of *Neospora caninum*

Neospora caninum (*N. caninum*) is associated with endemic and epidemic bovine abortion throughout the world (Dubey, 1999). Neonatal mortality and birth of feeble calves in cattle and a variety of species such as sheep and goats are other problems caused by *N. caninum*. With SVANOVIR® *Neospora*-Ab test you can accurately diagnose if the reproduction problems in a herd is caused by *N. caninum*. The assay enables evaluation on serum, plasma as well as milk samples (both individual samples and pools). Antibodies to *N. caninum* can also be detected in serum from aborted calves. The test's high specificity is needed in order to reliably differentiate *N. caninum* from other parasites, *e.g. T. gondii*. According to studies performed with the assay no cross reactions occurs with *T.gondii*, *S. cruzi*, *E. alabmensi* or *B. divergens* (Björkman *et al*., 1997).



Picture by Dubey 1999

SVANOVIR® *Neospora-Ab*, an indirect ELISA for detection of *Neospora caninum* in milk and serum from cattle and aborted calves

SVANOVIR® *Neospora*-Ab is based on an antigen presentation with membrane antigens incorporated into immune stimulating complexes ("iscom´s") that results in higher specificity of the assay. The test is applicable for surveillance and continuous testing for *N. caninum* as well as for declarations for freedom of disease. The tests ability to detect antibodies in both, milk and serum samples allows the parallel investigation of lactating cows, calves, bulls and dry cows. Antibodies to *N. caninum* can also be detected in serum of aborted calves.

PERFORMANCE CHARACTERISTICS | SVANOVIR® *Neospora*-Ab has been validated in several studies and demonstrated excellent performance with serum and milk samples. The test has been used in a study by Varcasia *et al*., 2005 including 624 milk samples from Italy where the ability of the test to screen for *N. caninum* in bulk milk samples and divide herds into different serological classes was demonstrated. The assay also showed a good correlation (k=0.941) in test results between serum and milk in samples from farms with confirmed abortion problems due to *N. caninum* and from farms without any clinical history of neosporosis. According to another study performed with the assay no cross reactions occurs with *T. gondii*, *S. cruzi*, *E. alabmensi* or *B. divergens* (Björkman *et al*., 1997). The assay can also differentiate between acute and chronic infection and this is of great value in the management of outbreaks (Björkman *et al*., 2003). The test also proved to perform much better than 2 competitors in a benchmarking test as seen in Table 2.

Table 2. Comparison of the SVANOVIR® *Nesospora*-Ab with an IFAT and two competitor's tests. Benchmarking, internal.

Serum samples, n= 121[a]	**Sensitivity**	**Specificity**	**Detection limit**
IFAT and in-house ELISA	n_{pos}= 45	n_{neg}= 76	
SVANOVIR® *Neospora*-Ab	91 %	99 %	1/128
Competitor 1	84 %	95 %	1/256
Competitor 2	82 %	94 %	1/32
Samples from [a]Swedish cattle population			

ASSAY OVERVIEW

SVANOVIR® *Neospora*-Ab

Species	Bovine, ovine and caprine
Samples	Serum/plasma, individuals and pools ≤10 Milk, individual and pools ≤50
Type	Indirect ELISA based on *Neospora caninum* iscoms (membrane antigen incorporated into immune stimulating complexes)

Article number	**Samples***	**Plates**	**Format**
104898	184	2	Strips

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

Boehringer Ingelheim Svanova
Veterinary Diagnostics

Located in Uppsala in the middle 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 diagnostics 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 products are manufactured and supplied according to the ISO 9001:2008 quality management system.

Boehringer Ingelheim has employees worldwide that work every day on the development of high quality vaccines, pharmaceuticals, nutraceuticals and diagnostic solutions to keep animals healthy. Our vision is to foster the health and well-being of mankind by contributing to an adequate supply of safe and nutritious food. Simply, we care about the health of humans and animals.

Today the Boehringer Ingelheim Svanova portfolio comprises over 30 different antibody detection ELISAs and two Penside tests, for direct detection of the infectious agents, supplied to more than 70 countries worldwide. We have sales partners in most of the world providing you products and services.

To find out more about our products and our company please visit www.svanova.com.

We are passionate about the health and well-being of humans and animals

Immunodiagnostics from Boehringer Ingelheim Svanova contribute to a global, adequate supply of safe and nutrious food

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