[ Workshop 6 ]

IBR eradication in the EU: success and pitfalls of the strategies, current status. The bulk tank as an innovative way of monitoring IBR.

Date: Friday, June 8th, 2012, 9.30 am - 1.00 pm.
Venue: Faculdade de Medicina Veterinária-UTL. Lisbon.
Control of infectious bovine rhinotracheitis (IBR) is now a target in more and more European countries. Diagnostic and vaccine tools are available and rather efficient to achieve this goal. However, Europe is not a homogenous entity: wide differences can be found with respect to husbandry, basic epidemiological situation and farming practices. This workshop aims to point out these differences with the intention to enrich our knowledge of IBR control. Intentionally IBR control and eradication are distinguished. The contributors of this workshop were invited from countries where IBR control is still running or even beginning. Keeping in mind the final achievement, i.e. IBR eradication, they have the best experience of the success and pitfalls of the control programs in use in their respective countries. The round table will enlarge the discussion to other European and non European countries and will be the forum to share experiences.

Etienne THIRY
Chairman

In Europe the cattle industry is moving towards eradication of BoHV1: The majority of our cattle population is currently implicated in some type of eradication activity against IBR, six countries are officially already IBR-free, and some territories (countries, regions) are very close to eradication. However some countries still have not taken any decision in this respect. At this stage, this type of seminars intend to promote the debate and exchange of experiences that will for sure be positive and interesting for all. This last point is actually very much in connection with the aim and spirit of the World Buiatrics Congress.

During the session the IBR strategies will be analyzed, and I am positive that will give a good overview of what is being done in some countries, and evidence the differences between the several programs which are being implemented. Finally, exploring what can be obtained from the bulk tank in this BoHV1 campaigns will be for sure worthwhile. The knowledge and experience of the speakers participating in this workshop is by itself a guarantee that the meeting will be rich in contents and debate, and hopefully it will be a good opportunity for the vets with a specific interest in IBR attending this workshop to update themselves in this topic. Thank you all, speakers and vets, for participating in this IBR workshop.

Santiago CASADEMUNT
Corporate Brand Manager HIPRABOVIS® - HIPRA

**AGENDA**

**Friday June 8th 2012**

9:30 am – 1:00 pm

Faculty of Veterinary Medicine, Lisbon

**Chairman: Etienne THIRY**

- IBR control and eradication in Europe: progress of the running programs (Etienne THIRY, U Liège, Belgium).
- Performances and pitfalls of IBR diagnostics (Jet MARS, GD, The Netherlands).
- The bulk milk sampling: only valuable with an increased detectability (Lourdes PORQUET, Hipra, Spain).
- Coffee break.
- IBR control: the German experience (Patricia KÖNIG, FLI, Germany).
- IBR control: the Italian experience (Stefano NARDELLI, IZT Veneto, Italy).
- Round table about serological diagnostics for IBR control.
IBR CONTROL AND ERADICATION IN EUROPE: PROGRESS OF THE RUNNING PROGRAMS

Etienne THIRY
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Infectious bovine rhinotracheitis (IBR) is a disease that can cause severe economical losses in livestock. Since the onset of the respiratory form of IBR virus (bovine herpesvirus 1; BoHV1) infection, live-attenuated and inactivated vaccines have been developed and they successfully controlled clinical consequences of the infection. In the early nineties, several European countries were in a favorable epidemiological situation and decided to control and eradicate the infection by a removal process without the use of vaccines: Norway in 1992, Denmark in 1993, Finland in 1994, Sweden in 1995, Austria in 1997 and the Italian province of Bolzano in 1998. The setting up of marker vaccines, based on the deletion in glycoprotein E (gE) gene, opened a new way for this IBR control, introducing an intermediate status of individual animals and herds, namely the gE minus status (meaning not infected but possibly vaccinated animals). The use of these vaccines made possible to start an IBR control leading to a further potential eradication in countries facing a higher prevalence of seropositive, and therefore likely latently infected, bovines: The Netherlands, Germany, France and Belgium were the first ones to initiate voluntary or mandatory programs and are now followed by other European member states. Concurrently, the European Union introduced rules for taking into account these new IBR status, and they are now known as referring as to Article 9 (approved IBR control program) and Article 10 (recognised IBR free status) of Directive 64/432/EEC. The combination of efficient diagnostic tools, gE and glycoprotein B (gB) ELISAs, and marker vaccines contributed to the success of these IBR control programs which is also based on the involvement of the farmers and animal health associations because these programs are not well (or not at all) supported by the national and regional governments.

So far, these programs successfully and significantly decreased the prevalence of BoHV1 (gE) seropositive animals. However, Europe is waiting for additional regions and countries joining the Bavarian German state which was recently officially declared IBR-free. This situation means that the implementation of an IBR control towards eradication is complex and time consuming. The removal of the last IBR positive animals remains a difficult task. The success of such an IBR control program depends on several points of concerns that are summarised here.

- The diagnostic tests are of good quality, but the sensitivity and predictive value of the negative result of gE ELISA tests make difficult the interpretation of some results, and especially at an individual basis. The presence of ruminant alphaherpesviruses highly related to BoHV1 and infecting other ruminant species, goat, buffaloes, red deer and reindeer can challenge the specificity of the serological tests. These ruminant species, including sheep, could be also potential reservoirs of BoHV1 infection. The sensitivity of the gB ELISA test can be also hampered by the sporadic appearance of seronegative latent carriers.

- The recommended schedule of repeated vaccinations was tested in several field and experimental studies. However, all currently marketed vaccines have not been evaluated by independent trials testing specifically their efficacy in BoHV1 eradication at a herd level. The dogma of the 6 month duration of immunity should be re-evaluated: is there a need to stimulate every 6 months the immune response of a mammal to keep a sufficient level of epidemiological protection at a population level?

- The efficacy of IBR eradication programs should be specifically assessed at the end of the program. At that time, a low prevalence can be stabilised by a decrease in IBR positive herds compensated by re-infected ones.

- Although highly mitigated by the in process control of vaccine manufacturing, the risk of live attenuated vaccine contamination with adventitious agents is still possible; the consequences can be disastrous: in the Netherlands, the IBR program was mandatory only in 1998-1999 and, due to such vaccine contamination event with a bovine viral diarrhoea virus, it was made voluntary since 1999.

- Accompanying measures are essential: good management and balanced financing of the program; biosecurity measures in the controlled farms; control of introduction into the herds; international (European), national and regional regulations; public and private incentives. These measures could be forgotten by focusing mainly on diagnostic testing and vaccination.
In the Netherlands a national IBR control programme was started in 1998.

In this voluntary control programme, several diagnostic assays to detect antibodies against BoHV1 infections are used. Mainly the gE ELISA is used, in individual serum samples, individual milk samples and in bulk milk samples. In addition, an in-house-PCR is used in nasal samples in case of (possible) outbreaks.

Validation data of these diagnostic assays will be presented. Sensitivity and specificity will be presented. The possibilities and limitations of the use of these diagnostic assays will be discussed: combination with vaccination strategy, sampling strategy, (how many how often), interpretation of results, comparison with other diagnostic assays, pitfalls, precautions.
THE BULK MILK SAMPLING: ONLY VALUABLE WITH AN INCREASED DETECTABILITY

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Introduction
The detection of antibodies in Bulk Tank Milk (BTM) provides an easy and cheap methodology for monitoring the health status of the herd. The application of this methodology to the detection of IBR-gE antibodies presents some limitations. Blocking IBR-gE-ELISAs have low sensitivity. So, IBR-gE-ELISAs are only capable of detecting positive tanks when the prevalence in the animals in production is greater than 15-20%. Under these conditions, gE detection in the BTM is not adequate for monitoring the tank and even less so for the classification of the farms. The objective of HIPRA is to develop and validate an IgG concentration method to increase the sensitivity of the IBR-gE detection systems in BTM sample.

Material and Methods
We have developed a simple methodology that in less than 60 minutes can concentrate up to 30 times the IgGs from a sample of 5.0 ml of BTM. To validate this methodology 17 dairy farms from the northwest of Spain with a known IBR status were selected (8 vaccinated and 9 non-vaccinated). From each farm we have obtained individual sera of all the animals in production and a sample of each tank. Taking into account the IBRgE results in sera, the true prevalence of each tank was calculated. Non concentrated and concentrated BTM samples were also analysed for gE antibody.

Results
The actual prevalence against gE, calculated from individual serum samples in the 17 selected farms, was: 0% (5 farms), 4%, 15%, 17%, 18%, 19%, 21%, 24%, 26%, 33%, 43% and 44% (2 farms). By using non-concentrated milk only 5 tanks were consistently detected as positive to gE. These tanks corresponded to the farms with the higher prevalence values (from 26% to 44%). By contrast, applying to the same samples the methodology of concentration of IgGs, all tanks were detected positive, even the lowest prevalence one. This methodology did not affect the 5 negative tanks that remained negative (Fig. 1).

Discussion
These results indicate that the proposed IgG concentration system does not affect the specificity of the ELISA but increases the sensitivity, having allowed in this study to detect tanks with the lowest prevalence (4%).

"CIVTEST® BOVIS IBRgE is not marketed in Portugal"
BoHV1 control in Germany started in 1986 as a voluntary program in numerous federal states. Farmers’ associations, breeding organizations, and public animal health foundations initiated a systematical survey of antibody prevalences, hygienic measures and vaccination campaigns.

BoHV1 control in Germany is based on two different strategies, which are mainly depending on the initial BoHV1 sero-prevalences. In herds, regions or federal states with low rates of BoHV1-infected animals, the so-called “conventional eradication” concept focuses on selection of BoHV1-seronegative animals without vaccination. In regions with high BoHV1-sero-prevalences, eradication is based on immunisation with glycoprotein E (gE)-deleted marker vaccines and the subsequent selection of marker-negative animals.

In the further course of the German eradication program, BoHV1 was classified in the year 1997 as a notifiable disease by the adoption of the “Legislation for the protection of cattle holdings from an infection with Bovine Herpesvirus Type 1”. Steady declining numbers of BoHV1 outbreaks are observed in Germany since 1998. BoHV1 control was intensified as an EU-official, compulsory and nationwide program in the year 2001. Due to the stringency and progress of the BoHV1 control program, Germany was granted Article 9 status in 2004. This status is coupled with additional trade guarantees and restrictions (European Union Directive 64/432/EEC). In 2007, the European Commission awarded the status “BoHV1 free” (Article 10) to the Bavarian districts “Upper Franconia” and “Upper Palatinate”, while in the last year whole Bavaria has received the Article 10 status as the first federal state in Germany (>99.8% BoHV1-free farms without vaccination).

At the end of 2010, nationwide 90.4% of the dairy and breeding herds in Germany were BoHV1 free (with or without vaccination), and in 6.3% of the herds eradication was still in progress.

Since the start of the BoHV1-control program, several millions of BoHV1 antibody assays are conducted yearly in Germany. Monitoring and status control are maintained in yearly sampling intervals. In the year 2010, approximately 3.8 million individual blood- or milk samples from about 71,400 holdings and approximately 293,000 bulk milk samples were serologically tested. An overview of the data for the last year is expected not before May 2012.

Efficient BoHV1 control requires a complex interplay of efficient diagnostics, consistent selection of reagents, stringent vaccination schedules, professional farm management, and effective hygiene- and quarantine measures. In conclusion, the progress as well as the main “lessons learned” from the German BoHV1-eradication program will be presented and discussed.
To date, the only Italian regions where compulsory IBR control programmes are ongoing are:
- The Bolzano province (since 2000 recognized as IBR-free according to Art. 10 Directive 1964/432/EEC)
- The Trento province and the Friuli Venezia Giulia region (IBR control programmes approved in 2009 according to Art. 9 Directive 1964/432/EEC)

These areas include only a minority (often small-sized) of the Italian breeding herds, whereas the largest ones (with the most Italian breeding population) are hosted in other regions, usually running IBR control programmes on a voluntary basis.

As a general rule, in the areas where voluntary programmes are ongoing, the situation is scattered; some improvements have been achieved at farm level, but all these areas are very far from approaching the eradication of the disease at the geographical level. In the high-density dairy areas (e.g. some provinces of Lombardia and Veneto region) marker vaccines are widely employed, but currently still some farmers keep using the cheaper (and often polyvalent) whole-virus vaccines, when their main economical product is represented by the milk rather than breeding animal production.

Considering the not IBR-free areas where a compulsory program is ongoing, the currently available data show that:
- over the years there is a regular decrease of the number of infected animals and farms, despite the fact that no economic reimbursement is foreseen for culling seropositive animals (which is not compulsory);
- most outbreaks occur without causing relevant clinical signs: the only finding is given by the seroconversion in IBR-antibody free animals;
- most outbreaks occur in larger farms having seropositive animals;
- significant gE seroconversions may occur inside marker-vaccinated herds, thus highlighting the need for not forgetting the direct prevention measures.

There are some practical difficulties in managing the compulsory, EU-approved IBR eradication programs, that have to be highlighted:
- Blood testing for gE antibodies in marker vaccinated animals does not have any possibility to be confirmed through supplementary diagnostic reactions: it is therefore very difficult to resolve single positive reactions arising in a group of gE negative animals e.g.
- Bulk milk testing for IBR antibodies suffers from two main drawbacks, i.e.:
  ▪ the size of the bulk milk pool: to the author’s knowledge no ELISA commercial kits are suitable for testing bulk samples of more than 50 cows: that causes practical difficulties when collecting samples in the frequently larger dairy farms;
  ▪ the absence of a commercial gE antibody bulk milk test, having an adequate sensitivity.
- The movement of buffaloes (Bubalus bubalis) is often restricted, because they result positive in the conventional IBR tests due to the infection caused by the cross-reacting herpesvirus BuHV-1, normally circulating in the buffalo population; to date, no discriminatory tests are available.
- Fattening farms often undergo many troubles when importing animals from foreign countries, because of the sanitary restrictions laid down by the decision EU 2004/558, which restrict the range of farms eligible for delivering fattening animals.
The prevalence of serum antibodies to bovine herpesvirus 1 (BoHV1) observed in several studies in Spain indicated the need to introduce a control program, specifically in the north-west regions, the main cattle producing areas in the country.

BoHV1 control programs were established during the biennium 2003-2004. The surveillance of cattle included both dairy and beef herds.

The main characteristics of the programs in Spain are: (1) voluntary (2) developed at regional level (not national) and (3) conducted through Sanitary Defense Associations (ADSG in its Spanish acronym). Each of the ADSG members included herds located in a specific geographic area. Thus, belonging to an ADSG is voluntary; but, once into the association, for it to be officially recognised and eligible for the subsidy scheme offered by the regional authorities, all the herds comprised have to carry out a BVDV, BoHV1, paratuberculosis and neosporosis control program. From the economical point of view, the subsidy scheme consists of: (1) support for the recruitment of veterinarian responsible for the programs, (2) support for the purchase of zoosanitary products related to program development (vaccines) and (3) reduction in the laboratory test prices.

Bovine ADSG began to be generalised in 2003-2004 mainly in the Cantabrian cornice, which concentrate the cattle census. Initially, efforts focused on (1) establishing the epidemiological situation of the different regions and herds involved and guidelines for herd monitoring, by means of serological analysis, (2) providing training programs for veterinarians and farmers and (3) controlling all purchased cattle. This was the basis for achieving a gradual reduction in the prevalence levels by gradually removing seropositive cattle and replacing them with seronegative animals. It must be considered that, in Spain, the use of conventional vaccines was widespread.

Although strongly recommended from the beginning, conventional non marker vaccines were banned from 2006-2007. Besides, from 2006-2007 on, bulk tank milk samples are used for monitoring dairies.

From 2011 on, farms classification program, according to their BoHV1 status, was initiated on the following basis:

1. free farm (without vaccination): herd in which all animals are seronegative for antibodies against the gB protein of the virus;
2. free farm with vaccination: herd in which all animals are seronegative for antibodies to the gE protein;
3. farm in control program: operation under study, establishing control or eradication program with either vaccination or not;
4. not classified farm: farm without diagnostic tests.

Galicia and Asturias (northwest) are the regions where control programs have reached a higher level of development and where more data have been generated. These two regions account for 52% of Spain’s bovine dairy population and 46.7% of their milk and 20.5% of their beef production quotas. The epidemiological baseline situation in both regions is shown in Table 1.
The number of ADSG increased in Galicia, from 33 in 2004 (that comprised 7.5% of the herds and 25% of the animals) to 75 at present (20% of the herds and 47% of the animals). In Asturias the trend was similar; from 21 ADSG in 2003 (5% of the herds and 9.4% of the animals) to 37 in 2010 (19% of the herds and 35% of the animals).

The current situation regarding BoHV1 control in these areas at herd and animal levels is presented in Table 2.

### Table 1. Prevalence of serum antibodies against the gB protein at the individual cow and herd level at the beginning of BoHV1 control programs.

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<th>Herd-level (%)</th>
<th>Animal-level (%)</th>
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<tbody>
<tr>
<td>Galicia (2004)*</td>
<td>65.3</td>
<td>36.9</td>
</tr>
<tr>
<td>Asturias (2003)</td>
<td>34.2</td>
<td>14</td>
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*data from dairy cattle

### Table 2. Prevalence of serum antibodies against the gB*/gE protein at the individual cow and herd level according to the last data available from the BoHV1 control programs

<table>
<thead>
<tr>
<th></th>
<th>Herd-level (%)</th>
<th>Animal level (%)</th>
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<tbody>
<tr>
<td></td>
<td>gB protein*</td>
<td>gE protein</td>
</tr>
<tr>
<td></td>
<td>Herd-level (%)</td>
<td>Animal level (%)</td>
</tr>
<tr>
<td>Galicia (2004)*</td>
<td>34.2**</td>
<td>50.9**</td>
</tr>
<tr>
<td>Asturias (2003)</td>
<td>46.6</td>
<td>33.7</td>
</tr>
</tbody>
</table>

*the use of the gB ELISA is kept by its higher sensitivity for diagnosis of non-vaccinated herds

**by bulk tank milk samples

The evolution was favorable, especially in regard to animal-level prevalence, considering that each year new herds are incorporated into the program. As older cows were culled, in many cases still vaccinated with conventional vaccines, a wide improvement is also expected at herd level.

The next steps should include decisions affecting the whole population of cattle, and not only herds in ADSG, primarily with regard to the ban of conventional vaccines and animal movement control.
Monitoring of gE antibodies in the milk tank

Concentrated on IBR

Regular high-detectability gE monitoring in the milk tank, through IgG concentration.
Doubly deleted gE-/tk- IBR live vaccine for cattle

The first doubly deleted (gE-/tk-)
IBR vaccine in the world

- No vaccine latency
- No vaccine re-excretion
- Genetic stability

MARKS A TREND

HIPRABOVIS® IBR MARKER LIVE


Target species: Cattle (calves and adult cows). Indications for use, specifying the target species: For the active immunisation of cattle from 3 months of age against Bovine Herpes Virus type 1 (BoHV-1) to reduce the clinical signs of Infectious bovine rhinotracheitis (IBR) and field virus excretion. Onset of immunity: 21 days after completion of the basic vaccination scheme. Duration of immunity: 6 months after completion of the basic vaccination scheme. Special precautions for use in animals: Vaccinate healthy animals only. Adverse reactions (frequency and seriousness): A slight increase in body temperature up to 1° C is common within 4 days following vaccination. Occasionally, an increase in rectal temperature up to 1.63º C in adult cows and up to 2.18º C in calves may be observed. This transient rise in temperature is spontaneously resolved within 48 hours without treatment and it is not related to a febrile process. A transient inflammation at the inoculation site is common in cattle within 72 hours post-vaccination. This slight swelling lasts for less than 24 hours in most cases. Vaccination might exceptionally cause hypersensitivity reactions. In such cases, an appropriate symptomatic treatment should be administered. Use during pregnancy or lactation: Can be used during pregnancy and lactation. Recommended vaccination programme: Cattle: from the age of 3 months onwards. The recommended initial dose is 1 injection of 2 ml of the reconstituted vaccine per animal. The animal should be revaccinated 3 weeks later with the same dose. Thereafter a single booster dose of 2 ml should be administered every six months. The method of administration is by intramuscular route, in the neck muscles. Reconstitute the lyophilised tablet with the entire contents of the enclosed solvent to obtain a suspension for injection. The solvent should be allowed to warm to a temperature between 15 ºC to 20ºC before reconstitution of the lyophilised tablet. Ovadose (symptoms, emergency procedures, antidotes), if necessary: No adverse reactions except those mentioned above were observed after the administration of a 10-fold vaccine dose. Withdrawal period: Zero days. Incompatibilities: Do not mix with any other veterinary medicinal product, except the solvent supplied for use with the veterinary medicinal product. Shelf life: Shelf life of the lyophilisate as packaged for sale: 2 years. Shelf life of the solvent as packaged for sale: 2 years. Shelf life after reconstitution: 6 hours. Special precautions for storage: Store and transport refrigerated (2° C - 8° C). Do not freeze. Keep the bottles in the outer carton in order to protect from light. Marketing authorisation holder: Laboratorios Hipra, S.A., Amer (Girona), Spain. Marketing authorisation numbers: 5 doses: EU/2/10/114/001 ; 25 doses: EU/2/10/114/002.

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