

Abstract Book

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Intra-uterine treatment with cefquinome of acute metritis in cows

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Acute metritis is due to a massive intra-uterine poly-bacterial infection (*E. coli*, *A. pyogenes*, *Prevotella spp.*, *Fusobacterium spp.*) occurring within about 2 weeks after calving. The disease results in impaired reproductive performances, and may be life-threatening if the infection extends beyond the uterus.

Cefquinome, a fourth generation cephalosporin already used in the veterinary field (Cobactan), has a broad spectrum of activity adapted to treat the bacterial species present during metritis.

The suitability of a new cefquinome intra uterine formulation for treatment of acute metritis was investigated in two field trials in which we tested placebo and various dosages of cefquinome: 300 mg, 600 mg, 900 mg and 1200 mg.

Cows within 2 weeks post partum and with a foul smelling, red-brown uterine discharge, were included. Intra-uterine treatment was performed on the day of inclusion after clinical examination. Cows were examined again about 3 days later and were re-treated in case symptoms had not improved.

The clinical examination was repeated at approximately 7 and 21 days after admission. Cows not improved at day 7 were considered as treatment failures and were not

evaluated anymore at day 21. At day 21 cows were either classified as cured (absence of pathological uterine discharge) or as failures (chronic endometritis).

The key parameter to evaluate performance of the treatment was the day 21 cure rate. The following values were obtained: 42% - 49% for placebo (n=19 and n=45); 31% for 300 mg (n=16); 47% - 68% for 600 mg (n=17 and n=79); 74% - 75% for 900 mg (n=19 and n=80); and 67% for 1200 mg (n=86).

Treatment with the three highest doses of cefquinome provided a benefit over placebo. An optimal efficacy was obtained at 900 mg ($p=0.0032$). Further increase of the dose to 1200 mg did not result in an improved efficacy. Analysis of secondary parameters such as rectal temperature, evolution of uterine discharge aspect, and uterine evolution confirmed this picture.

Examination of the ovaries at day 21 by rectal palpation showed that 45% (n=78) of the cows treated with 900 mg cefquinome had palpable follicles, in comparison to 25% (n=40) for the placebo group ($p=0.0354$).

These results demonstrate that intra-uterine administration of a formulation containing 900 mg of cefquinome treats efficiently acute metritis, which positively influences ovarian activity resumption.

Clinical efficacy of Cobactan® LA 7.5% in the treatment of naturally occurring bovine respiratory disease (BRD) in Europe

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Introduction

The objective of this study was to assess the efficacy of a flexible dose regimen of Cobactan® LA 7.5% in the treatment of BRD under field conditions, in comparison with another cephalosporin, Excenel® RTU.

Materials and Methods

The study was carried out following the requirements of Good Clinical Practice (GCP) on farms in the Netherlands, France and Germany as a randomized, blinded field study. Study animals were mainly Black Pied cattle, Holstein Friesian and Charolais weighing from 38 up to 509 kg. Veal calves, rearing calves in dairy farms and animals in pre-fattening units were enrolled on Day 0 when showing signs of BRD (rectal temperature $\geq 40^{\circ}$ C and impaired breathing and impaired general attitude). Animals were weighed and treated either with Cobactan® LA 7.5% 1-2x (48 hours apart) with a dose of 2.5mg/kg body weight or with Excenel® RTU with a dose of 1 mg/kg body weight daily on 3-5 consecutive days. The necessity of repeated treatment was based on the clinical response and the severity of symptoms. Assessment of efficacy was made on Day 12 \pm 1 (overall treatment success). Animals showing signs of BRD between Day 5 and Day 12 \pm 1 after intermediate treatment success on Day 5 were defined as relapses.

Results

A total of 194 animals were treated and examined according to the protocol. The overall treatment success was comparable in the two treatment groups with 74.0% (71 of 96) for Cobactan® LA 7.5% and 77.6% (76 of 98) for Excenel® RTU (non-inferiority test: $p=0.0021$). The relapse rate was 6.6% (5 of 76) for Cobactan® LA 7.5% and 13.6% (12 of 88) for Excenel® RTU (non-inferiority test: $p<0.0001$). There was no significant difference between treatments with regard to the course of rectal temperatures between Day 0 and Day 5 and in the daily weight gain. 36 animals (38%) in the Cobactan® LA 7.5% group were treated only on the day of inclusion while 60 animals (62%) were re-treated after 48 hours. Among the Excenel® RTU treated animals 64 (65%) received up to 3 daily treatments and 34 animals (35%) received 4 or 5 daily treatments. Animals treated less than 3 times were treatment failures ($n=3$). No drug related adverse reactions were observed.

Conclusion

Cobactan® LA 7.5% given at a dosage of 2.5 mg/kg b.w. cefquinome in 1-2 injections is equivalent in overall treatment success rate in BRD when compared to Excenel® RTU given at a dosage of 1 mg/kg b.w. in 3-5 injections. The treatment with Cobactan® LA 7.5% is well tolerated and tends to result in a decreased number of relapses.

Pharmacokinetic and pharmacodynamic aspects of Cobactan® LA 7.5% in cattle

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Introduction

The objective of this review is to describe and analyse pharmacokinetic (PK) and pharmacodynamic (PD) aspects of Cobactan® LA 7.5% for its use in cattle to treat bovine respiratory disease (BRD).

Methods

In a study to determine the plasma pharmacokinetic profile of the active ingredient cefquinome following administration of Cobactan® LA 7.5% in cattle, three groups of eight animals each were treated subcutaneously once at dose levels of either 1.25 mg, 2.5 mg or 5 mg cefquinome /kg bw. Equal numbers of male and female animals weighing between 92.5 and 132.0 kg were used. Blood samples were taken pre-treatment and in intervals between 0.5, and up to 72 hours after treatment. Cefquinome samples were analysed by HPLC/UV with a LOQ of 0.030 µg/ml. PK parameters like C_{max} , T_{max} , AUC_{all} and time above minimum inhibitory concentration ($T > MIC$) for the respiratory pathogens *P. multocida* and *M. haemolytica* were determined using Pharsight WinNonlin Professional, utilising non-compartmental analysis.

Results

The pharmacokinetic behaviour of cefquinome tended to be linear for the doses administered. Absorption occurred rapidly. For the medium dose of 2.5 mg/kg bw, a mean plasma concentration of 0.266 µg/ml was reached within 30 minutes, corresponding to 8.3X the historically steady MIC_{90} of ca. 0.032 µg/ml for *P. multocida* and *M. haemolytica*. C_{max} , T_{max} , AUC_{all} for the 2.5 mg/kg bw dose were 1.070 µg/ml, 6.0 hrs and 14.072 hr*µg/ml; $T > MIC_{90}$ for both respiratory pathogens was 38.74 hrs, and concentrations higher or equal to 10X MIC_{90} were maintained for 18 hrs.

Discussion

These data demonstrate a combination of pharmacological qualities of cefquinome formulated as Cobactan® LA 7.5%: a rapid systemic availability with plasma concentrations far above the MIC_{90} of *P. multocida* and *M. haemolytica* within 30 minutes of subcutaneous administration, and prolonged systemic availability above MIC_{90} at a dose of 2.5 mg/kg bw. Other in vitro data demonstrate that for Pasteurellaceae a short exposure of approx. 1 hr to cefquinome at 10X MIC followed by an exposure to 0.5X MIC leads to a significant and prolonged inhibition of bacterial re-growth and bactericidal activity. In summary, the PK/PD profile of Cobactan® LA 7.5% is the basis for rapid and extended systemic activity of its bactericidal and time-dependent active ingredient cefquinome against major bovine respiratory pathogens in diseased cattle.

Early assessment of the outcome of treatment with Cobactan® LA 7.5% on naturally occurring bovine pneumonia

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Introduction

The objective of this evaluation was to analyze the predictability of successful treatment of naturally occurring bovine pneumonia with Cobactan® LA 7.5%.

Treatment failure in cattle suffering from pneumonia often leads to irreversible lung tissue damage. Therefore it is essential for the person applying the treatment to assess the efficacy as early as possible.

Material and Methods

The analysis was based upon a randomized, blinded multi-centred field study on farms in the Netherlands, France and Germany, following the principles of Good Clinical Practice (GCP).

Animals showing signs of bovine respiratory disease (BRD) (rectal temperature $\geq 40^{\circ}\text{C}$ and impaired breathing and impaired general attitude) were enrolled into the study (Day 0). Animals were treated with Cobactan® LA 7.5% once or twice (48 hours apart).

The necessity of 2nd treatment was based on the clinical response and the severity of symptoms after 48 hours. Animals presenting a rectal temperature $< 40^{\circ}\text{C}$, a normal to slightly impaired breathing and a normal general attitude were not re-treated.

An assessment of treatment efficacy was made on Day 5.

Results

96 animals were treated with Cobactan® LA 7.5% and completed the study according to protocol. An assessment of the clinical response was made on Day 2 based on data of 91 animals (5 severely affected animals had to be withdrawn before Day 2).

Treatment success rate of all animals on Day 5 was 79.2% (76 of 96). The success rate on Day 5 among animals treated once was 93.5% (29 of 31). 60 of 91 (65.9%) animals received a second treatment after 48 hours.

It was observed that a sub-group of animals (12 of 60) with a rectal temperature $\leq 39^{\circ}\text{C}$, normal to slightly impaired breathing and normal to slightly impaired general attitude responded well to the second treatment and displayed a success rate of 100% (12 of 12) on Day 5.

Success among the animals more severely affected on Day 2 was 72.9% (35 of 48).

Conclusion

The study demonstrates that animals showing an improvement of clinical signs on Day 2 after treatment are cured without a second treatment. For those not clinically improved on Day 2 and therefore treated again, the severity of clinical signs is a reliable predictor for the treatment effect on Day 5.

Efficacy of Cobactan® LA 7.5% against bovine respiratory disease caused by *Histophilus somni*: Pharmacokinetic and pharmacodynamic data

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Introduction

Cefquinome, a broad spectrum cephalosporin, has gained market authorization in 1994 for the treatment of bacterial bovine pneumonia. *Histophilus somni* is one of the contributing pathogens in bovine respiratory disease. The relevance of this pathogen however, is probably commonly underestimated as it is very difficult to culture. *H. somni* is also associated with lethal septicaemia, myocarditis and thrombotic meningoencephalitis. Therefore a rapid and effective treatment is mandatory to prevent animal losses. The objective of this study was to confirm the in-vitro efficacy of Cobactan® LA 7.5% against infections caused by *H. somni*.

Materials and Methods

Pharmacodynamic data: A total of 25 strains of *H. somni* recently isolated from diseased cattle were tested according broth microdilution method as described in CLSI (formerly NCCLS) guideline M 31-A2.

Pharmacokinetic data: Cobactan® LA 7.5% was administered subcutaneously at a single dose of 2.5mg/ kg body weight cefquinome to 4 male and 4 female calves. Cefquinome plasma levels were determined by HPLC/UV technique before injection and at 0, 0.5, 1, 2, 4, 8, 12, 18, 24, 30, 36,

42, 48, 54, 60, 66 and 72 hours after injection with a level of quantification of 0.003 µg/ml.

Results

All tested strains were highly susceptible to cefquinome with MICs below or equal to 0.016 µg/ml.

Plasma levels of cefquinome exceed MIC values within 30 minutes after injection. Maximum concentration of 1.07 µg/ml (C_{max}) was reached after 6 hours, being more than 60 times the MIC₉₀ of 0.004µg/ml. Cefquinome concentrations remain above 0.016µg/ml for at least 42 hours.

Conclusions

For cephalosporins the common parameter for the assessment of efficacy is time above MIC. After subcutaneous injection the plasma levels reach therapeutic levels in less than 30 minutes and persist above MIC₉₀ for more than 42 hours. The plasma concentrations are high enough to cover more than 85% of the 48-hour treatment interval. From these data it can be concluded that Cobactan® LA 7.5% has rapid and prolonged systemic activity, and can therefore be recommended for treatment against *H. somni* infections in bovine respiratory disease.

Survey of cefquinome susceptibility of *Pasteurella Multocida* and *Mannheimia haemolytica* isolated from diseased cattle in Europe from 1994 to 2005

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Introduction

Cefquinome, a fourth generation cephalosporin, has gained market authorization in 1994 for the treatment of bovine pneumonia caused by *P. multocida* and *M. haemolytica*. From that time on, Intervet implemented a surveillance program to monitor the susceptibility of relevant bacteria against cefquinome.

Materials and Methods

A total of 1142 bacterial strains (650 *P. multocida* and 492 *haemolytica*) were isolated from bovine respiratory disease in Europe from 1994 to 2005. Minimum inhibitory concentrations were determined using broth dilution method either according to Deutsches Institut für Normung (DIN) (1994-1999) or to Clinical and laboratory Standards (CLSI) (2000-2005). In addition, minimum bactericidal concentrations (MBC) were determined for the pathogens isolated during 2000-2005. MIC₅₀ and MIC₉₀ were determined annually for each strain.

Results

For *P. multocida*, in the defined time period, MIC₅₀ and MIC₉₀ remained equal to or below 0.032 µg/ml, with the exception of the MIC₉₀ found in the first two years of testing, i.e. ≤ 0.063 µg/ml in 1994 and 0.25 µg/ml in 1995.

The MIC₅₀ and MIC₉₀ for *M. haemolytica* were equal to or below 0.032 µg/ml, except for the second and third year of testing (1995-1996), where the MIC₉₀ was 0.063 µg/ml.

For *P. multocida*, MBC₅₀ was 0.032 µg/ml during 2000-2004 and decreased to 0.016 µg/ml in 2005. MBC₉₀ was 0.5 µg/ml during 2000-2004 and 0.063 µg/ml in 2005.

For *M. haemolytica*, MBC₅₀ was 0.016 µg/ml decreasing to 0.008 µg/ml in 2005 and MBC₉₀ was 0.063 µg/ml during 2000 - 2004 and 0.032 µg/ml in 2005.

Conclusions

These results demonstrate that cefquinome shows an unchanged high activity against the main respiratory pathogens *P. multocida* and *M. haemolytica* isolated during the last ten years in Europe. Bactericidal activity against both bacteria was shown at very low concentrations.

Efficacy of Cobactan DC in cows during the dry period and after calving

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The objective of this study is to evaluate the efficacy of Cobactan DC, a broad spectrum cephalosporin, in comparison to a positive control, Cepravin® Dry Cow, in a clinical field trial.

This controlled, randomized and partly blinded study was conducted in accordance with EMEA/CVMP guidelines. 4 farms were selected on a Bulk Milk Somatic Cell Count <200.000 cells/ml in the preceding 3 months and/or a high (>10-20%) prevalence of clinical mastitis caused by gram-negative mastitis, in the dry period or in the first 3 months after calving. On these farms, late lactation cows were dried off with either Cobactan DC or Cepravin Dry Cow.

Efficacy of treatment was assessed at the quarter level and based on bacteriological cure rate, prevention rate, new infection rate and clinical mastitis rate. Mastitis rate was assessed during the dry period and the first 60 days in milk. All other parameters were assessed at calving and 5-7 days post-calving. All parameters were analyzed in relation to the duration of the dry period.

Efficacy of treatment was measured on 121 cows dried off with Cobactan DC and on 115 cows with Cepravin Dry Cow. The overall bacteriological cure of Cobactan DC was 87.6%, significantly non-inferior ($p=0.0038$) to

Cepravin® Dry Cow (83.8%). Non-inferiority of Cobactan DC could also be shown for the majority of isolated gram positive pathogens (96.9%), including CNS. Unfortunately, conclusions on efficacy on Gram-negative pathogens such as *E. coli* could not be drawn due to the low incidence of isolated cases (3.1%). This was probably due to the high prevalence (58%) of high SCC cows at dry off. Prevention rate, new infection rate and clinical mastitis rate were comparable too for both products, 84.8%, 14.0%, 16%, respectively for Cobactan DC and 85.3%, 15.3%, 17.9%, respectively for Cepravin Dry Cow.

For cows exceeding a 63 day dry period, Cobactan DC showed a prevention rate similar to the control (86.6% vs. 86.8% respectively) and a considerably higher, however not significantly higher (85.7%), bacteriological cure rate compared to the control (72.7%). For both products, there were no adverse events related to treatment.

This study shows that the curative and preventive efficacy of Cobactan DC is equivalent to Cepravin Dry Cow. When the dry period exceeded 63 days, the prevention rate in the Cobactan DC group was similar to that of Cepravin and Cobactan DC tended to have a higher bacteriological cure rate.

Monitoring of cefquinome susceptibility against mastitis isolates collected from diseased cattle in Europe from 1994 to 2005

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Introduction

Cefquinome, a fourth generation cephalosporin, is marketed within Europe since 1995 for the treatment of bovine mastitis. Intervet monitored the susceptibility of cefquinome over time against major mastitis pathogens. The study covers the period 1994 to 2005.

Material and Methods

A total of 2228 bacterial strains (1737 Gram positive and 491 *E.coli*) have been isolated from mastitis in Europe from 1994 to 2005. Minimum inhibitory concentrations were determined with broth dilution method either according to DIN (1994-1999) or to CLSI (2000-2005) with a two fold increase in the antibiotic concentration range. MIC₅₀ and MIC₉₀ were determined each year or time period of strain collection.

Results

Overall, no increase in the MIC₅₀ and MIC₉₀ was observed for all target pathogens tested over the last ten years.

S.uberis constantly showed a MIC₅₀ ≤0.03 µg/ml and a MIC₉₀ of 0.12 µg/ml.

Depending on the year, *S.aureus* and coagulase negative *Staphylococci* MIC₅₀ were either 0.25 or 0.5 µg/ml and MIC₉₀ either 0.5 or 1 µg/ml.

For *S.dysgalactiae*, the MIC₅₀ and MIC₉₀ were always between 0.063 and ≤0.03 µg/ml

Susceptibility of *S.agalactiae* increased over the period of strain collection. The MIC₅₀ remained ≤0.03 µg/ml but the MIC₉₀ of 0.12 to 0.25 µg/ml from 1995 to 1997 was ≤0.03 µg/ml from 1998 to 2005.

For *E.coli*, the MIC₅₀ was either ≤0.03 or 0.06 mg/ml and the MIC₉₀ either 0.06 or 0.12 µg/ml.

Conclusion

These results demonstrate that cefquinome has a high activity against the main mastitis pathogens i.e. *S.aureus*, *S.uberis*, *S.agalactiae*, *S.dysgalactiae*, coagulase negative *Staphylococci* and *E. coli*, and that the susceptibility has not changed over the last ten years in Europe.

A comparative field trial of Mastiplan (cefapirin and prednisolone) and Peracef (cefoperazone) for the treatment of bovine clinical mastitis

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Introduction

The objective of this trial was to assess the efficacy of a Mastiplan formulation (300 mg cefapirin and 20 mg prednisolone, 4x at 12h interval) and Peracef (100 mg cefoperazone, 2x at 24h interval) for the treatment of clinical mastitis in lactating cows. A multi-centre randomised positive controlled field trial was conducted in accordance with guidelines (EMA/CVMP/344/99) in Germany and Hungary in 1997-1998.

Materials and Methods

The efficacy of treatment was determined by the bacteriological cure rate *i.e.* elimination of pathogen identified at day 0, and the clinical cure rate *i.e.* affected quarter with normal milk and no signs of clinical mastitis (swelling, induration or pain of the udder) at days 14 and 21.

In total 202 cows were treated with Mastiplan LC and 204 with Peracef.

Results

Main pathogens at the day of admission were *Staphylococcus aureus* (17%), *Escherichia coli* (16%) and *Streptococcus uberis* (13%). The pathogens were equally distributed between the two treatment groups. No bacteria were isolated for 10.3% of the clinical cases. The mastitis cases included were equally divided over the treatments with respect to country and pathogens involved.

The overall bacteriological cure rate was 39% for Mastiplan LC and 35% for Peracef and was better in Germany than in Hungary. This difference was not observed for the overall clinical cure rate which was 61% for Mastiplan LC and 59% for Peracef.

For Mastiplan LC the bacteriological cure rates were 24%, 44% and 47% for staphylococcal, *E. coli* and streptococcal mastitis respectively. For Peracef the bacteriological cure rates were 37%, 43% and 31% for staphylococcal, *E. coli* and streptococcal mastitis respectively.

Clinical cure rates ranged from 47% (*E. coli* mastitis) to 82% (staphylococcal mastitis) for Mastiplan LC and from 47% (streptococcal mastitis) to 77% (staphylococcal mastitis) for Peracef. Before treatment, swelling, induration and pain of the udder were higher in the Mastiplan LC treatment group ($P=0.007$) and the scores declined faster than those in the Peracef group.

Statistical modeling by means of a non inferiority test ($\alpha=0.05$; $\delta=20\%$), showed that Mastiplan LC was not inferior to Peracef.

No suspected adverse reactions were observed for either of the products and both products were considered as safe.

Conclusion

The efficacy of Mastiplan LC was similar and not significantly different to that of Peracef for the treatment of clinical mastitis in lactating cows.

A comparative field trial of Mastiplan LC and Synulox for the therapy of bovine clinical mastitis

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Introduction

The efficacy of Mastiplan LC for the treatment of clinical mastitis in cows was investigated in a multi-centre randomised positive controlled field trial. The study was conducted in accordance with the guidelines (EMEA/CVMP/344/99) in the Netherlands, France, the UK and Hungary in 1997-1998.

Materials and Methods

Lactating cows with clinical mastitis in one quarter were treated intramammarily with a Mastiplan LC formulation containing 300 mg cefapirin sodium and 20 mg prednisolone (4x with a 12h interval) or with Synulox containing 200 mg amoxicillin, 50 mg clavulanic acid and 10 mg prednisolone (3x with a 12h interval).

The efficacy of treatment was determined by the bacteriological cure rate i.e. elimination of pathogen identified at day 0, and the clinical cure rate i.e. affected quarter with normal milk and no signs of clinical mastitis (swelling, induration or pain of the udder) at days 14 and 21.

Results

In total 260 cows were treated with Mastiplan LC and 257 with Synulox. Main pathogens at the day of admission were *Streptococcus uberis* (22.6%), *Escherichia coli* (14.3%)

and *Staphylococcus aureus* (10.4%). The pathogens were equally distributed between the two treatment groups. No bacteria were isolated for 24.0% of the clinical cases. The overall bacteriological cure rate was 49.4% for Mastiplan LC and 40.7% for Synulox. The overall clinical cure rate was 74.7% for Mastiplan LC and 65.1% for Synulox.

Bacteriological and clinical cure rates of streptococcal mastitis were 60.0% and 77.2%, respectively for Mastiplan LC and 54.5% and 71.4%, respectively for Synulox.

Bacteriological and clinical cure rates of *E. coli* mastitis were 58.6% and 75.0%, respectively for Mastiplan LC and 42.5% and 59.5%, respectively for Synulox.

Bacteriological and clinical cure rates of staphylococcal mastitis were 25.7% and 73.0%, respectively for Mastiplan LC and 27.5% and 63.6%, respectively for Synulox.

Statistical modeling by means of a non inferiority test ($\alpha=0.05$; $d=20\%$), showed that Mastiplan LC was not only non-inferior to Synulox but even superior.

No suspected adverse reactions were observed for either of the products.

Conclusion

From this study it is concluded that Mastiplan LC is safe and more effective than Synulox for the treatment of clinical mastitis in lactating cows.

A randomised, controlled field trial to compare the efficacy of Mastiplan LC and Synulox in the treatment of bovine clinical mastitis

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Introduction

Efficacy and safety of Mastiplan LC in the treatment of clinical mastitis in lactating cows was assessed under field conditions in France, Spain and Italy in 2005. This clinical trial was multi-centred, non-blinded, positive controlled and randomised.

Materials and Methods

355 lactating cows were randomly allocated to a treatment group and treated intramammarily in the infected quarters with either Mastiplan LC (cefapirin and prednisolone; 4x at 12h interval) or Synulox (amoxicillin/clavulanic acid and prednisolone; 3x at 24h interval).

Bacteriological status of milk, temperature, general condition, milk quality, udder firmness, induration, and pain were evaluated at day0 (inclusion day), day+15 and day+22. General condition, milk quality, udder firmness, induration, and pain, were evaluated at each milking from day0 to day+4.

The bacteriological cure rate was defined at the quarter level by the elimination of the pathogen present at day0 in samples collected at day+15 and day+22. The clinical cure was defined at the cow level by the return to normal of the general condition, the udder and milk quality at day+15.

Results

The main pathogens isolated at day0 were *Strep. uberis* (18%), *E. coli* (14%), *Staph. aureus* (5%), *Strep. agalactiae* (4%) and *Strep. dysgalactiae* (4%).

Treatment of clinical mastitis resulted in an overall bacteriological cure rate of 53% for Mastiplan LC and 45% for Synulox. The clinical cure rate was 62% for Mastiplan LC and 52% for Synulox.

($\alpha=15\%$ $\delta=0.05$) Statistical modeling by means of a non inferiority test (showed that Mastiplan LC was non-inferior to Synulox. A trend of superiority of Mastiplan LC was demonstrated for overall bacteriological and overall clinical cure ($P=0.0002$ and $P<0.0001$, respectively) and for bacteriological cure of infections caused by Gram-positive bacteria ($P=0.002$), Staphylococci ($P=0.008$) and Streptococci ($P=0.022$).

A rapid and complete reduction in scores of inflammation (modified milk, swelling, firmness and pain of udder) is observed during treatment and is comparable in both treatment groups. No suspected adverse reactions due to treatment were observed.

Conclusion

Percentages of cure rates of Mastiplan LC were higher than those obtained with Synulox. Statistical analysis showed that Mastiplan LC is not inferior with a trend of superiority as compared to Synulox. Treatment leads to a rapid and complete reduction of signs of inflammation.

Antibiotic susceptibility by the disk diffusion method: bovine respiratory pathogens

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Objectives and Methods

All microorganisms tested were collected by field veterinarians in 17 French departments, in due course of Intervet Technical Assistance to the field vets on respiratory diagnostic.

78% of biological materials were obtained from Trans Tracheal Aspiration on recently infected animals with clinical signs of respiratory disease and 21% were obtained from lung tissue at necropsy.

Cattle included in this survey were aged between 7 days and 2 years. Samples were sent to field laboratories in 2004. Inhibition diameters for cefquinome and 18 other antibiotics frequently used for respiratory disease were measured by the disk diffusion method for 59 strains of *Pasteurella multocida*, 16 *Mannheimia haemolytica*, 5 *Arcanobacterium pyogenes*, 2 *Haemophilus somnus* and 1 *Pseudomonas aeruginosa*. Antibiotics tested and critical diameters (mm) are the following: streptomycin (13-15), gentamycin (14-16), ceftiofur (17-21), cefquinome (18), amoxicillin (14-21), spiramycin (19-24), spectinomycin (20), tylosin (13-18), tulathromycin (9-12), tilmicosin (11-15), florfenicol (15-19), colistin (15), flumequin (21-25), enrofloxacin (17-22), danofloxacin (17-22), marbofloxacin (14-18), TMPS (10-16), tetracyclin (17-19) and doxycyclin (17-19).

Results

Generally speaking, *M. haemolytica* was less susceptible to the tested antimicrobials than *P. multocida*. Excellent sensitivity was observed for cefquinome. Most I/R results were obtained for aminoglycosides, macrolides (spiramycin, tylosin) and tetracyclines.

Strains of *Haemophilus somnus* were classified Intermediate or Resistant uniquely to spiramycin, and Sensitive to all other antibacterials tested. The respiratory strain of *Pseudomonas aeruginosa* was Sensitive only to cefquinome, colistin and marbofloxacin.

Conclusion

With respiratory disease, pathogenic bacteria isolated by Trans Tracheal Aspiration (TTA) are more interesting than the ones isolated by Bronchio Alveolar Lavage (BAL), the latter being most probably commensals. Results obtained with spiramycin are not expected and most probably reflect inadequacy of the method or breakpoints for this macrolide. Results of antibiotic sensitivity is similar to other studies conducted with cefquinome since its first use in France in 1995. Clinical efficacy of the cefquinome-based product has already been confirmed by numerous clinical field trials and has been supported by pharmacokinetic data as well.

Neonatal colibacillosis in calves: comparison of two injectable antibiotic treatment in addition to rehydration

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Objectives

Cefquinome has demonstrated its diffusion in case of inflammation in different tissues and secretions: bronchial secretions, cerebrospinal fluids, mastitis milk. Danofloxacin is known to penetrate the digestive tract, even with no inflammation.

The purpose of this multicenter, randomised and controlled study was to evaluate the benefit of these two molecules as injectable treatment of neonatal colibacillosis.

Methods

Calves < 11 days with signs of moderate dehydration (<10%), born from non vaccinated dams, were included on D0. The same day, faecal sampling was performed. These samples were checked for pathogenic *E. coli*, cryptosporidia, rotavirus Ag, coronavirus Ag or K99 *E. coli* Ag. All calves were rehydrated per os (D0 and D1) and were randomly selected for injection with either danofloxacin (6 mg/kg/j SC on D0) or cefquinome (2 mg/kg/j IM on D0, D1, D2). Additional IV rehydration (1 L/animal on D0) was performed when necessary. Clinical cure was judged by veterinarians using a Total Clinical Score (TCS) given on D0, D1, D3 and D7. This TCS is the sum of 6 scores noted from 0 (normal) to 3 (worst) concerning attitude, appetite, demeanour, dehydration, stools consistency, abdomen contractions. Cure was

defined as an improvement of TCS on D3 or D7 compared to D0, with body temperature <39.5°C and with, at most, a single individual score between 1 and 3.

Results

Finally, 78 calves on average 4 days old (48 ± 6 kg) were included in the analysis (42 and 36 cases in each group). IV rehydration (85% of calves) and clinical signs on D0 were comparable in both groups. Most frequent serotypes of *E. coli* were found to be CS31A, K99 and F41. Only 7 faecal samples out of 78 had unknown serotypes of *E. coli*, without associated agent (virus or cryptosporidia). TCS and each of the individual 6 scores, including body temperature, were similar in both groups ($p > 0.05$, ANOVA). The differences of TCS observed on Day 7, in favour of the cefquinome treated calves, were not statistically significant. The proportion of clinical cure (around 70% on D3 and D7) was not statistically significant (Chi-square).

Conclusion

This trial concludes that the treatment of diarrhea calves with rehydration fluids, combined with modern antibiotics like cefquinome or danofloxacin that have a high capacity of diffusion, effectively controls the negative impact of neonatal colibacillosis

A multicenter, randomised, field trial comparing Dexafort® and Voren suspension® for the treatment of bovine hyperketonemia

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Hyperketonemia is a common disease in dairy cattle, caused by a negative energy balance that occurs often after calving. Hyperketonemia can affect milk production and reproduction performance and be associated with a decreased non-specific immunity. Both clinical and sub clinical hyperketonemia result in increased concentrations of ketone bodies in the tissues and milk of cows.

Administration of glucocorticoids and glucose infusion are widely used in the treatment of bovine hyperketonemia in veterinary practice and its efficacy has been demonstrated in experimental and field studies.

The objective of this trial was to assess under field conditions the efficacy of Dexafort® (Intervet) as a treatment for hyperketonemia in dairy cows and to compare the efficacy with a reference product Voren suspension® (Boehringer Ingelheim).

The trial was designed as a randomised multicentre clinical trial and was performed according to Good Clinical Practice in veterinary practices in France and Hungary.

134 cows with hyperketonemia included to the trial were randomly treated either with a single dose of Dexafort® (0.2 ml/10 kg bw) or with Voren suspension® (0.2 ml/10 kg bw) and an infusion of glucose. Plasma concentrations

of ketone bodies (b-hydroxybutyrate) were assessed at the time of inclusion (day 0, i.e. D0) and on days D3, D5 and D7 post treatment. Treatment success was defined as plasma concentrations b-hydroxybutyrate on D7 within the normal limits (i.e. <1.2 mmol/L).

The results of 123 cows were possible to interpret: 63 of which received Dexafort and 60 of which received Voren suspension. The success rate (percentage of animals with plasma concentration of b-hydroxybutyrate <1.2 mmol/L on D7) of 70% was noted for Dexafort and 33% for Voren suspension. Recovery rate of animals treated with Dexafort was 37% on day 3 and 63% on D5. In case of Voren suspension recovery rates of 15% and 23% were achieved on day 3 and Day 5 respectively.

The percentage of animals relapsing (as defined by a b-hydroxybutyrate plasma concentration dropping below 1.2 mmol/L and then increasing above that threshold) was 10% in the Dexafort group and 15% in the Voren group.

This trial demonstrated the superiority of Dexafort over Voren suspension for the treatment of hyperketonemia in dairy cattle both in total recovery rate and the speed of the recovery process.

Therapeutic efficacy of an amitraz + chlorpiriphos association against *Boophilus microplus* in naturally-infected cattle

Results of six trials

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Boophilus microplus is the main cattle ectoparasite in Brazil. Difficulties have been faced to control this parasite due to its resistance to nearly all existing chemical groups with acaricide action. The use of combination of actives with different mechanisms of action is one of the strategies suggested to control the resistance. This study aimed to evaluate the anti-ixodid efficacy of a new formulation containing 50% chlorpiriphos (as emulsionable concentrated) plus 50% amitraz (as wettable powder) spray, in comparison to the use of these actives separately or commercial compounds containing organophosphate-pyrethroid associations against *B. microplus* in naturally infested cattle. Six trials were carried out in different regions of Brazil, with the inclusion criteria of the presence of 4.5 to 8.0 mm *Boophilus microplus* female ticks on the animal's left side. The chlorpiriphos + amitraz association presented high efficacy levels, above 90% (91.99% to 100%) on the 3rd day post-treatment (DPT),

except in one trial. On the 21st DPT, also except in one trial, this formulation has presented efficacy percentage above 95% (97.78% to 100%), showing an extended protection period. In a general isolated chlorpiriphos presented high efficacy but a reduced residual period, as expected for an organophosphate. A reduced amitraz efficacy was noticed in two farms, indicating resistance to this active. The cattle treated with the combination containing 60% ethion + 8% cypermethrin (1:1000) and 1.2% flumetrine + 16% coumaphos (1:400) showed a higher number of *Boophilus microplus* ($P < 0.05\%$) than the ones treated with the evaluated formulation. The results obtained in the six trials presented demonstrated that the amitraz+chlorpiriphos combination presented higher activity against *B. microplus* than amitraz and chlorpiriphos used separately, even in the case of the strains of ticks that are resistant to amitraz.

Endectocide activity and weight gain among cattle treated with a formulation containing 1% abamectin + ADE vitamins

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The success of cattle farming is based on the adoption of technological achievements, preferably in an integrated way in order to optimize the performance. Concerning to sanitary measures, parasite control must be emphasized as a fundamental factor to maintain economic competitiveness. The efficacy of a formulation containing 1% Abamectin + ADE* was established in four trials, and compared to other endectocides and an untreated group.

In the first trial, the experimental formulation presented similar anti-ixodid efficacy (>95%) to 1% Ivermectin + ADE**, but with lower *Boophilus microplus* infestation levels from the 28th day onwards. In the second trial, the formulation was as efficacious as 1% doramectin against *Dermatobia hominis* larvae. Both medications presented an efficacy rate of 100% until the end of the study (post treatment day (DPT) 91). The anthelmintic efficacy of the

formulation was checked in the third assay, via coprologic (EPG) and necroscopic exams, with 100.0% efficacy against five of the eight species of nematodes that have been diagnosed, a similar result to that obtained with 1% ivermectin + ADE** in the same trial. Body weight development of recently weaned calves was analyzed in a 4th trial, comparing 1% Abamectin + ADE*, 1% Ivermectin + ADE**, and 1% Abamectin LA**. It was noticed that the average weight gain after 60 days, among cattle treated with 1% Abamectin + ADE differed ($P < 0.05$) from the gain obtained in the other groups, with a difference of 16.17 kg; 7.34 kg, and 6.06 kg, compared to control group animals and to those treated with 1% Ivermectin + ADE and 1% Abamectin LA, respectively.

From these data, it can be concluded, that 1% Abamectin + ADE is a very efficacious formulation.

Improvement of fertility after vaccination with an inactivated bovine viral diarrhoea virus (BVDV) vaccine in BVDV-infected dairy herds

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Infection with the bovine virus diarrhoea virus (BVDV) shortly before or after insemination reduces the conception rate. The underlying mechanisms are not yet completely understood. Modern commercially available BVD-vaccines are generally tested for their capacity to prevent infection of the bovine foetus, but data on the efficacy to reduce the negative effects of a BVDV infection on the conception are hardly available.

Therefore, we have investigated, whether vaccination with Bovilis® BVD can improve the reproductive performance of breeding cattle in BVDV sero-positive dairy husbandries. 17 dairy herds in the UK were included in a field study. At admission, all cows were screened for BVDV specific antibody and seronegative animals were tested for BVD virus. Bulk milk samples were tested at bi-monthly intervals to monitor potential field virus circulation in the herds. The cows were ranked according to age and calving dates and allocated to the vaccinated or unvaccinated control group. The vaccination was performed according to the manufacturer's instructions. The effect of vaccination was judged by the following parameters: number of

inseminations per pregnancy, pregnancy rate to first insemination, overall pregnancy rate and intercalving period.

Only one herd showed a marked increase in the bulk tank antibody titre, indicative of a rise from less than 5% to more than 65% seropositive animals. This corresponded with the birth of a virus-positive calf early in the course of the trial. The bulk milk antibody titres started and remained high in the other herds indicating a high proportion of seropositive animals throughout. BVDV virus was detected in two other farms. Fertility rate to first service was statistically significantly lower ($p < 0.05$) in the control group compared to vaccinated group in 3 farms, 2 of which had identified virus positive animals in contact with the milking herd. Further evidence of improved fertility was also recorded in vaccinated animals from these three herds. There was no evidence of effect on fertility in the other herds.

It was concluded, that vaccination with Bovilis BVD improved the reproductive performance in herds with an endemic BVDV infection and virus circulation.

Seroprevalence of BVDV may be different for the various age groups on dairy farms - results from a field survey on 17 farms in the west of England

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Infection with bovine virus diarrhoea virus (BVDV) causes considerable losses to the cattle industry, especially in breeding herds. For practical reasons, epidemiological surveys are often performed by antibody testing of milk tank samples.

However, in dairy herds in the UK, breeding animals of different ages are often kept in separate groups before calving and subsequent entry to the dairy herd. This may lead to groups of differing serostatus on an individual farm.

We have performed a serosurvey of different age categories of breeding females on 17 different dairy farms in the West of England. Only farms with no history of vaccination were included in the study.

The cattle were segregated into three distinct age cohort groups: bulling heifers sampled around the time of first breeding, pregnant replacement heifers and lactating

cows in the milking herd. The animals were blood sampled and tested for the presence of BVDV specific neutralising antibodies. Bulk milk tank titres from the milking herds were also quantified.

In any age group, cattle appeared to be either predominantly seropositive or seronegative. The seroprevalence of the three different age groups within a farm was more often than not similar but on 6 of the 17 farms distinctly different seroprevalence profiles existed between the age groups. Therefore, the bulk antibody level or the seroprevalence in any one age group is a relatively unreliable predictor of the susceptibility of other age groups, particularly before joining the milking herd. This finding has implications for control policy. Decisions regarding control measures should be taken on a herd by herd basis and separately for the milking herd, the pregnant heifers and the bulling heifer age groups.

A field safety and efficacy study for the concurrent use of Bovilis® BVD and Bovilis® IBR marker in dairy cattle

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The aim of the study was to assess safety and the efficacy of the concurrent use of an inactivated BVDV vaccine (Bovilis® BVD) with a modified live gE deleted BoHV-1 vaccine (Bovilis® IBR marker) in dairy cattle. Eighteen heifers aged 6 to 8 months old seronegative both for BoHV-1 and BVDV were randomly attributed to one of the three groups: 5 animals were vaccinated with Bovilis® IBR marker; 5 animals were vaccinated with Bovilis® BVD and 8 animals were vaccinated with Bovilis® IBR marker and Bovilis® BVD in two different injection sites. Both vaccines were administered intramuscularly (2 ml each dose) in the neck. Vaccinations were repeated 28 days later. For the purpose of this study Bovilis® IBR marker was administered twice although the vaccine has been demonstrated to be efficacious after a single administration. In order to evaluate the safety aspects, fever, anorexia, depression and local reactions were evaluated for 5 days after vaccination. Blood samples were collected at beginning of the study, 4 weeks (booster) and 7 weeks (21 days after the 2nd vaccination). Samples were tested for virus neutralising (VN) antibodies against BoHV-1 and BVDV, and by ELISA test for BoHV-1 gE and BVDV NS2-3 (p80/120) antibodies. Mean logarithmic (log

10) virus neutralising antibody (VN) titres for BoHV-1 and BVDV were calculated, and a statistical evaluation of the data was made.

Neither local nor general reactions were observed in any of the three groups. The two products demonstrated to be safe when administered simultaneously at two different injection sites. All the animals vaccinated with Bovilis® IBR marker seroconverted for BoHV-1 after priming vaccination. All the animals vaccinated with the BVDV vaccine showed seroconversion for this virus after the booster vaccination. There were no significant differences between the three groups for the immune response against BoHV-1 and BVDV. No BoHV-1 or BVDV field infections occurred during the study as demonstrated by the fact that all the heifers remained negative for BoHV-1 gE and for BVDV NS 2-3 antibodies. In this study, Bovilis® BVD administered to naïve BVDV animals did not elicit detectable level of NS2-3 antibodies after two shots. Our data also confirm the immunogenic activity of Bovilis® IBR marker after one single dose. In conclusion, the concurrent administration of Bovilis® IBR marker and Bovilis® BVD at separate injection sites is safe and induces a consistent immune response.

Cross reaction varies considerably between different BVDV vaccines

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Bovine Virus Diarrhoea Virus (BVDV) is a pestivirus responsible for considerable economic losses in cattle throughout the world. The broad antigenic diversity of circulating BVDV strains sets high demands on the vaccines. The extent of cross-protection afforded by a vaccine can not be predicted e.g. on the basis of the genome sequence, but has to be determined experimentally.

In this study, we have compared two commercially available inactivated BVDV vaccines for their cross-reactivity against different BVDV strains. Vaccine 1 contains the BVDV Type 1a strain C86 (Bovilis® BVD) and Vaccine 2 contains the BVDV Type 1a strain KY 1203nc (Bovidec).

Ten calves that were seronegative and non-cytopathic pestivirus free were included in the study. At about 1 year of age, 5 randomly selected animals were vaccinated with vaccine 2 and the second group of 5 animals was vaccinated with vaccine 1. The vaccinations were performed according to the manufacturer's instructions

and repeated at a 4 or 3 weeks interval, respectively. Blood samples were taken prior to the first vaccination and 3 weeks after the second dose of vaccine was applied. They were tested for BVDV neutralising antibody titres against four heterologous BVDV strains representative for the BVDV types 1a, 1b, 2a and 2b.

All animals responded to the vaccination by the development of BVDV neutralising antibodies. Both vaccines induced antibody titres reacting with all four strains tested. Overall, the titres measured against the two BVDV type 1 strains were higher than against the BVDV type 2 strains. Moreover, the animals vaccinated with vaccine 1 developed higher antibody titres than vaccine 2.

From these results, it can be concluded, that inactivated BVD vaccines vary substantially in their extent of cross-reaction with a broad panel of BVDV strains. Bovilis® BVD was found to have a broad antigenic activity.

Efficacy of an inactivated bovine virus diarrhoea vaccine 12 months after vaccination

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Bovine Virus Diarrhoea Virus (BVDV) is a pestivirus responsible for considerable economic losses in cattle throughout the world. The most effective method for controlling the economic losses due to BVDV infection is to control the birth of persistently infected calves following transplacental transmission of the virus. This together with protection against foetal losses are both achievable through vaccination. Cell-free viraemia has been described as the main determinant in transplacental BVDV transmission to the developing foetus. A laboratory trial was therefore carried out to evaluate the capacity of Bovilis® BVD to prevent cell-free viraemia during a 12 month period.

Eleven BVDV naive (seronegative and non-cytopathic pestivirus free) were chosen for the study. At about 4 months of age, 6 randomly selected animals were vaccinated with Bovilis BVD according to the manufacturer's instructions. The vaccination was repeated after 4 weeks. One year after the vaccination all eleven animals were infected intranasally with a pool of 12 non-cytopathic pestivirus isolates. The animals were observed for clinical signs typical for BVDV infection. White blood cell counts

were recorded; nasal swab samples, serum and buffy coat cells were titrated for BVD virus. The BVDV neutralising antibody titres were followed throughout the study.

All five control calves became febrile, whereas pyrexia was not observed in the vaccinated calves. However, nasal discharge was observed in both experimental groups. Four out of five unvaccinated calves, but none of the vaccinated group developed a transient but significant decrease in white blood cell counts. Virus isolations from nasal swabs clearly showed a significantly reduced virus shedding in the vaccinated calves compared with that shed by unvaccinated animals. BVD virus was recovered from the buffy coat cells of all five unvaccinated animals and from 4 out of 6 vaccinated animals but the calves yielded virus on one occasion only. Most importantly, none of the six vaccinated calves were positive for virus in serum whereas significantly all five control calves, yielded virus in the serum for 3 to 5 days.

From these results, we conclude that Bovilis BVD vaccine affords complete protection against serum viraemia and therefore against transplacental BVDV infection for a duration of at least 12 months.

Suitability of an inactivated BVD vaccine for BVD eradication under Swiss field conditions

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Infection with the bovine viral diarrhoea virus (BVDV) has been recognized as a serious problem for the Swiss cattle industry. Elimination of the persistently infected (PI) animals from a farm and subsequent vaccination are the key factors in BVDV eradication on farm-level without any official support. The efficacy of a vaccine against the circulating field isolates is of crucial importance to avoid fetal infection with BVDV by contact of virus-free animals by contact with animals with unknown status, as it is quite often happen in Switzerland in trans humanence (Alpage).

An observational study was performed in a Swiss dairy herd with a history of BVD problems. PI-animals were removed followed by herd vaccination with an inactivated vaccine Bovilis® BVD-MD. The herd was followed for two years using commercially available tests for detection of BVD virus and BVD antibodies.

One PI animal was detected during the initial screening

and a second PI calf was born one month after completion of the basic vaccination. Both animals were removed from the farm. In the 24 months following the culling of the second PI calf, no further cases of PI calves were detected in the herd. The animals were grazed on common pastures, causing a known risk for infection. The control measures can therefore be considered successful.

In the second part of the investigation, the cross-reactivity of the vaccine with recent Swiss field isolates (n=5) was studied *in vitro*. Sera from animals vaccinated with Bovilis® BVD-MD were shown to have high antibody titres against the homologous vaccine strain C86 (BVDV type 1a). Also the heterologous titres against the Swiss field isolates were satisfactory.

These data indicate, that the commercially available inactivated vaccine Bovilis® BVD-MD should be taken in to account as a valuable tool for BVD prevention in the Swiss cattle herds as long as there is no federal support for an eradication program.

Control of BVDV infection through identification and removal of persistently infected (PI) animals and vaccination with an inactivated BVDV vaccine

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The control of the Bovine Viral Diarrhea Virus (BVDV) infection, foresees the identification and removal of the persistently infected (PI) animals and/or vaccination. Some inactivated vaccines, including Bovilis® BVD (Intervet), have proven protection against transplacental infection of the foetus, preventing the birth of new persistently infected (PI) calves. Aim of this study was to investigate the possibility to eradicate BVDV from an Italian commercial dairy farm. The eradication program consisted of the identification of BVDV seronegative animals, detection of PI animals and application of a vaccination scheme to protect the animals from reproductive disorders and to prevent the transplacental infection. In March 2003, all animals were tested for non structural BVDV antibodies (NS2-3), with an ELISA test. Antibodies against NS2-3 were detected in sera for 64.5% of the tested animals. The seronegative animals were then tested for viremia, with an ELISA antigen test. A positive result was confirmed by a second positive sample and the virus collected was cultivated on MDBK. Three PI animals, 2 calves and 1 heifer, aging > 5 months of age, were identified. For management reasons, these three PI animals, were not removed but remained

in the herd for other 16 months, until September 2004, when they were slaughtered. In this period at least one of these PI animals was in contact with pregnant animals, presenting a high risk of BVDV infection. In April 2003 all animals, aging more than 6 months, were vaccinated intramuscularly (2ml), twice at 28 days apart, with an inactivated BVDV vaccine (Bovilis® BVD-Intervet). A 6 months booster schedule was applied (the replacements were vaccinated twice at 6 month of age). Till October 2005, all the new born calves were regularly tested for the presence of viremia, and all the animals in the herd were tested every 6 months for BVDV NS2-3 antibodies. The percentage of animals positive to the BVDV NS2-3 antibodies remained stable for all the observation period. Despite the fact that the three PI animals remained in the herd for about 16 months, shedding virus, no further PI animals were detected after the introduction of the vaccination procedure. In conclusion, the data obtained in this study confirm the efficacy, demonstrated in previous studies, of Bovilis® BVD in preventing the transplacental infection of bovine foetus by BVDV.

Potential to use a conventional inactivated Bovine Viral Diarrhoea vaccine as marker vaccine

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Vaccines that enable differentiation between infected and vaccinated animals, the so called DIVA or marker vaccines have been developed for the bovine and porcine herpesviruses (IBR marker vaccines, Aujeszky's disease virus vaccine) and the classical swine fever virus. In the later, a single protein of the virus serves as subunit vaccine. Attempts to develop similar vaccines for another pestivirus, the bovine viral diarrhea virus (BVDV) failed due to the lack of efficacy. Since BVDV marker vaccines would be a very valuable tool in eradication programs, the possibility to use existing (inactivated) vaccines as a marker should be evaluated. As shown previously antibody levels against the non structural (NS) proteins of the virus are below or just above the limit of detection in animals vaccinated with inactivated vaccine Bovilis BVD and high in animals that have experienced a field virus infection. However, it has never been established, whether animals which are vaccinated against BVDV develop NS specific antibodies after subsequent field virus infection. In this study, five calves seronegative for BVDV were vaccinated twice at a 4-weeks interval with Bovilis BVD.

Three weeks after the second vaccination, the animals were infected intranasally with a BVDV field isolate. Blood samples were taken at different time points throughout the study and tested for BVDV neutralising antibodies. The antibodies against the NS3 protein were determined in 4 different ELISA systems.

As expected, all animals were tested negative for NS3 specific antibodies until second vaccination in all four test systems. At the time of challenge and one week later one or two animals gave positive results in three systems. One of them was tested doubtful in the fourth test one week after challenge. From two weeks after challenge onwards, all four ELISA's gave positive results.

From these results it can be concluded, that the inactivated vaccine Bovilis BVD has properties of a marker vaccine when an appropriate antibody NS3 ELISA is applied: after vaccination antibody levels are low or undetectable, but the vaccination does not interfere with the development of antibodies against NS3 after subsequent field virus infection.

Success of Bovine Viral Diarrhoea control by means of removal of persistently infected animals and vaccination

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The aim of this study is to prove the efficacy of a program in the control of BVD in farms where PI animals were detected, based on the removal of these and subsequent vaccination of the herd with an inactivated vaccine against BVD (Bovilis® BVD).

The test was carried out on 4 milking herds in the Northwest of Spain. The total number of controlled animals was 384.

All animals were tested using an ELISA that detects antibodies (ab) against the BVDV non structural p80 protein (Ingezin BVD, Ingenasa Spain), and those that gave a negative result were tested with another ELISA that detects BVDV p80 protein antigen (Pourquier Institute, France). One PI was identified in each herd, which was eliminated previous to vaccinating the whole herd with Bovilis® BVD, following the manufacturer's instructions. Serum samples taken before the colostrum intake were tested for antigen (p80 ELISA Pourquier Institute, France). These same animals were tested for seroconversion against p80 at the age of 6 months and then periodically every 6 months to see if they presented a seroconversion to p80.

The abortion rate in each of the herds in the 12 months prior to the date of vaccination was calculated and

compared to the 12 months after this.

Initial seroprevalence before vaccination among the animals that lodged with the PI animals was 81.9%. Of the 119 animals born after vaccination, none was PI and only 3 animals (2.5%) were positive to ab against p80 at some time points. These 3 animals had received multiple vaccinations (4, 4, and 6 respectively) before obtaining a positive result to p80 ab. These results indicate that the control method used was efficient in avoiding new PI births in the herd.

The average number of abortions on the 4 farms in the 12 months prior to vaccination was 10.31% against 6.11% in the 12 months after. In the 12 months prior to vaccination cows were 1,8 times more likely to abort than in the 12 months after starting vaccination (odds ratio = 1.77; 95% confidence interval: 0.78, 4.07).

Abortion rate in the first quarter of gestation was even reduced from 4.12% to 1.67 % after vaccination (odds ratio = 2.54; 95% confidence interval: 0.60, 12.27).

The elimination of PI animals and vaccinating with Bovilis® BVD are efficient methods to control the birth of new PI animals in the herd besides reducing the percentage of abortions after vaccination, mainly those that occur in the first quarter of gestation.

Monitoring of BHV-1 seroprevalence in a dairy cattle herd vaccinated with a BoHV-1 modified live marker vaccine: eradication in progress

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The development of gE deleted BHV-1 marker vaccines allows to differentiate antibodies induced by vaccination or by field infection. BHV-1 eradication programs, based on vaccination with marker vaccines and culling of animals that are seropositive for gE BHV-1 at the end of the reproductive career, are currently being implemented in Italy. Under field conditions we monitored the progress in the eradication of BHV-1 infection in a commercial dairy herd of about 280 animals vaccinated with a modified live marker vaccine (Bovilis® IBR marker - Intervet). Late in autumn of 2002, 212 animals out of the 280 were tested for gE BHV-1 antibodies with a commercial ELISA test. The gE seroprevalence was 22.6%. In April 2003 a vaccination program with the attenuated live Bovilis® IBR marker vaccine was started. All animals from the age of 4 months onwards were vaccinated, intramuscularly with one dose of 2 ml. Thereafter vaccination was repeated every 6 months and all the new-born calves were regularly vaccinated (2ml intramuscularly) at 4 months of age. Colostrum was collected from BHV-1 gE seronegative cows to feed to the newborn calves. Biosecurity measures namely introduction of only gE- BHV-1 animals, restriction of movement of people and vehicles

and use of disposable tools were applied. All the animals were sampled every 6 months to evaluate the gE BHV-1 antibody status. The seroprevalence was calculated for different groups: calves, heifers and cows. The gE seroprevalence in animals older than 5 months of age decreased progressively during the observation period: 17.9% on April 2003, 16.5% on October 2003, 16.1% on April 2004, 10.1% on October 2004, 12.3% on April 2005 and 8.2% at the last control on October 2005. The increase of the % of gE positive animals observed in April 2003, is due to the erroneously administration of colostrum from gE positive cows to 5 calves. These animals were found to be gE negative at the subsequent control. During the observation period, no respiratory/reproductive outbreaks referable to BHV-1 infection were noticed. Moreover no new gE positive animals were detected during the monitoring period, indicating that no reactivation of latently infected animals had occurred. In conclusion, the practice of vaccination with a BHV-1 live marker vaccine, implementation of biosecurity measures and the progressive culling of gE positive cows is an effective method to reduce gE prevalence in a herd during a BHV-1 eradication program.

Results from a controlled comparative study of two different vaccination schedules with Bovilis® IBR Marker

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The bovine herpesvirus 1 (BHV-1) is the causative agent for infectious bovine rhinotracheitis (IBR). This illness causes significant economic loss in the cattle industry worldwide and is countered through widescale vaccination.

The aim of this study was to establish the efficacy of a live IBR marker vaccine (Bovilis® IBR marker) after a primary course consisting of a single or double application.

Twenty-five BHV-1 seronegative calves at 2 weeks of age were randomly attributed to five groups (n=5). Calves in groups 1 and 4 were vaccinated intranasally (IN), and those in groups 2 and 5 intramuscularly (IM). Group 3 served as unvaccinated control. One month after the first vaccination, a second dose of vaccine was given to calves in groups 4 (IN/IN) and 5 (IM/IM). Six months after the second vaccination, all animals were subjected to a challenge virus infection with a pathogenic field isolate of BHV-1. The animals were observed for clinical signs typical for IBR. Nasal swab samples were titrated for BHV 1 virus and sera for the BHV-1 neutralising antibody (VN).

All animals developed BHV-1 specific VN titres after the first vaccination. The average titres were slightly higher in the IM groups. The IN re-vaccination did not result in a booster reaction, but three out of five calves in the IM/IM

group reacted with a significant increase in the antibody titres. As expected, titres declined in time. At the time of challenge, VN titres had dropped below the detection limit in some of the animals in the IN, IN/IN and IM group. Solely in the IM/IM group all animals were still seropositive at the time of challenge. All vaccinated animals developed an anamnestic immune response after challenge. The vaccinated calves only developed mild nasal discharge and no significant febrile reactions, irrespective of the route or schedule of vaccination. Unvaccinated calves in contrast were markedly affected by febrile IBR. The amount and the duration of challenge virus shedding in nasal mucus were significantly reduced in all four vaccinated groups as compared to the unvaccinated calves. There was however no significant difference due to the four vaccination schedules.

It is therefore concluded that a single administration of the live BHV-1 marker vaccine used in this study administered either by the IN or IM route to calves at two weeks of age does not differ in efficacy from the two dose vaccination schedule. The tested vaccination regimes conferred protection for at least 6 months.

Seroprevalence of bovine herpesvirus-1 after vaccination with a live marker vaccine in Hungary

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The development of Bovine Herpesvirus type 1 (BHV-1) gE-deleted vaccines allows discrimination between vaccinated and infected animals which makes them key tools in a BHV-1 eradication program. A national eradication program began in Hungary in 2002. At the same time a serological survey was started on farms that had either not implemented any BHV-1 control measures or had used conventional non-marker vaccines. Up to October 2005, more than 8700 samples were tested from 147 farms distributed all over Hungary. Most of the farms were dairy with an average size of 500 (range:80-1,800). After the initial BHV-1 serological evaluation the modified live Bovilis® IBR marker vaccine was applied as follows: In herds with a low seroprevalence all cattle from the age of 5 months onwards were primary vaccinated once intramuscularly vaccination was repeated every 6 months. In herds with high sero-prevalence, it was advised to vaccinate the calves intranasally from the age of 2 weeks onwards, repeat the vaccination by the intramuscular route by the age of 4 months and thereafter booster every 6 months. A second sero-prevalence study by means of gE ELISA was carried out 18-36 months after the start of the vaccination program targeting mainly the young age group, The following categories of animals were defined:

calves (0-6 (mostly 3-5) months of age, thus still having some MDA), maiden heifers (6 to 15-18 months of age), pregnant heifers (age about 18-26 months) and cows.

The data demonstrate that the seroprevalence for BHV-1 field virus decreased after vaccination in all categories. The effect of vaccination in the calves group (46% seropositive animals before vaccination and 40% after) was probably biased by the residual MDA. In the three older age groups, the number of BHV-1 positive animals clearly decreased after vaccination (maiden heifers: from 23% to 8%, pregnant heifers: from 41% to 7 % and cows: from 78% to 22%).

Data were available from 15 farms, to follow the seroprevalence directly in one or more age categories. The number of farms which were positive for BHV-1 in a certain age category decreased from six to two (out of nine) for the heifers, five to three (out of seven) for the pregnant heifers and from ten to three (out of ten) for the cows.

These data demonstrate, that vaccination with Bovilis® IBR marker live according to the recommended schedule, is a very efficacious way for eradicating BHV-1 from large scale cattle farms in Hungary.

Tailor made vaccination regime for control of bovine infectious rhinotracheitis (IBR) virus using live and/or inactivated IBR marker vaccines

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Vaccination against the infectious bovine rhinotracheitis (IBR) with marker vaccines which contain the glycoprotein E (gE) deletion mutants of the bovine herpesvirus type 1 (BHV 1) as vaccine strain is widely applied to control the disease. In contrast with other cattle vaccines, IBR marker vaccines are available both, live and inactivated. This exceptional situation offers the opportunity to develop different vaccination regimes adjusted to the needs of an individual farm situation namely: only live vaccine, only inactivated vaccine or first live vaccine followed by inactivated vaccine. Bovilis® IBR marker (live BHV-1 marker vaccine) and Bovilis® IBR marker inac (inactivated BHV-1 marker vaccine) contain the same GK/D strain. The efficacy of those two vaccines has been demonstrated for the vaccination protocols in which only live or inactivated vaccines are used. A trial was carried out to assess if the immunological response induced by Bovilis® IBR marker inac after an initial primary course with either Bovilis® IBR marker or Bovilis® IBR marker inac was comparable. Twelve approximately three months old calves, which were negative for antibody specific for BHV 1 were included in

an experiment using two licensed vaccine. A first group of six randomly selected animals was vaccinated twice with Bovilis® IBR marker inac via the intramuscular route at a four weeks interval. The second group received one dose of Bovilis® IBR marker live together with the first vaccination of group 1. Seven months after the (first) vaccination, all calves were re-vaccinated with a single dose of the inactivated vaccine. Blood samples taken at strategic time points during the study were tested for virus neutralizing antibody specific for BHV 1. The re-vaccination seven months after the (first dose of the) basic vaccination induced a rapid and marked increase in virus neutralizing antibody regardless, which product was used at the primary vaccination. This indicates an anamnestic response which supports the efficacy of one single dose of Bovilis® IBR marker inac to boost the immune response after priming with Bovilis® IBR marker live or inac. The implication of these results for the development of tailor made vaccination schedules adjusted to the farmer's needs and wishes are discussed.

A live BHV-1 marker vaccine is not shed after intramuscular vaccination

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Live marker vaccines against bovine herpesvirus type 1 (BHV-1) infections have proven to be important tools in eradication programs. It is well known that BHV-1-vaccinated animals excrete vaccine virus when the vaccine is applied via the intranasal route. However, few information is available concerning intramuscular (IM) use of that kind of gE-deleted marker vaccines.

Therefore, we have performed a study, to establish, whether animals vaccinated IM with a live BHV-1 marker vaccine (Bovilis® IBR marker live) become viremic and / or excrete vaccine virus with nasal discharge.

Five head of cattle, seronegative for BHV 1 at the start of the study were included in this experiment. The animals were vaccinated once with an overdose via the IM route. Nasal swab samples were taken daily for 11 days. Blood samples were taken three times a week during the first two weeks and then once a week until four weeks after vaccination. The nasal swab samples and the blood

samples were tested for BHV -1 in a virus infectivity assay. In addition, a polymerase chain reaction (PCR) specific for BHV-1 DNA was performed on the blood samples. BHV -1 neutralising antibody titres were determined in the sera taken prior to the vaccination and four weeks after immunisation.

All animals were successfully vaccinated as judged by the development of BHV -1 neutralising antibodies. However, all nasal swab samples were tested negative for vaccine virus, and all blood samples were found negative for BHV-1 virus and BHV -1 specific DNA.

From these data it can be concluded that the vaccine virus was not excreted with nasal discharge after IM vaccination and no viremia of vaccine virus could be detected.

Therefore, it is recommended to apply the tested BHV-1 marker live vaccine by the IM route in situations where it is undesirable that the vaccine virus is excreted.

Spreading of bovine herpesvirus type 1 in an infected herd can be efficiently controlled by vaccination - a case study

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The economic impact of infections with the bovine herpesvirus type 1 (BHV-1) is generally accepted. Following the development of efficacious BHV-1 marker vaccines, national BHV-1 eradication programs were initiated in a number of countries. According to the German BHV-1 decree, vaccination with a marker vaccine can be made compulsory for a BHV-1 infected herd in an area with a low disease prevalence.

Following the high tide of the Elbe in 2002, the milking herd stable needed to be evacuated and re-placement heifers/cows were purchased from different sources. Among those, a considerable number of animals were (latently) infected with BHV-1, resulting in a seroconversion rate up to 28.2% in the original milking herd.

A herd vaccination program with the live vaccine Bovilis® IBR marker was started in January 2003. Calves were vaccinated intranasally at 2 weeks of age and re-vaccinated at 3 months of age by the intramuscular route. To cows booster vaccination was given locally during late pregnancy about 3 weeks before the anticipated time of

calving to reduce the risk of reactivation. Additionally the following management measures were taken to reduce virus spread: BHV-1 infected animals were marked and separated from the negative animals during calving and where possible for any other handling. As far as possible, replacement heifers are recruited amongst the progeny of the herd.

According to the regulations as laid down in the BHV-1 decree, the herd was monitored serologically.

During the first year of the vaccination program, some new seroconversions were detected in all age groups and housing units, but in the following years, the seroprevalence of BHV-1 gE in the production herd (milking cows and heifers) decreased dramatically from 31.7% in Q1 2003, to 23.1% in Q1 2004 and 5.9% in Q4 2005.

It is expected that the BHV-1 eradication in this herd will be completed in 2006, 3 years after the start of the eradication program in this herd.

Evaluation of the anamnestic response of BRSV and PI-3 component of Bovilis® Bovipast RSP after a single booster at 3, 6 or 12 months post vaccination

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Respiratory disease due to infection with bovine respiratory virus (BRSV), Parainfluenza type 3 virus (PI3) and/or *M. haemolytica* is a major problem in young cattle and accounts for important losses in the industry. Vaccination against these agents is regularly carried out in young calves to prevent the clinical signs. Some of those animals may require receiving a booster administration. The purpose of this study was to assess and to compare the anamnestic response measured in virus neutralizing (VN) antibody titres against the BRSV and PI3 components of the vaccine Bovilis® Bovipast RSP after administration of a single dose booster vaccination at different times after the initial two doses primary course.

A field study was performed on 9 different farms in Norway with no history of recent BRSV infection. A total of 132 calves with low initial BRSV VN antibody titres were included in the study. The animals were allocated to one of four different treatment groups. The first group (A) served as unvaccinated control to monitor a concurrent field virus infection. The animals attributed to groups

B, C and D were given a primary vaccination with Bovilis Bovipast® RSP consisting of two doses of the vaccine. After 3 (group B), 6 (group C) or 12 months (group C), they received a single dose of the product.

The single booster vaccination given at different time intervals after the primary vaccination course induced a similar anamnestic response against BRSV in all three vaccinated groups. Moreover, the BRSV antibody titres after the single booster vaccination were similar to those after the second dose of the primary vaccination course. The primary vaccination course as well as the single booster vaccination induced high PI3 antibody titres that remained on high levels for at least 7.5 to 12 months in all three vaccinated groups.

From these results it can be concluded that the administration of the primary course induced a strong memory effect. The single application of Bovilis® Bovipast RSP as long as 12 months after the primary vaccination is sufficient to induce a protective immune response.

A single vaccination with an inactivated Bovine Respiratory Syncytial Virus vaccine affords protection in calves with maternal antibody

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Respiratory disease due to infection with bovine respiratory virus (BRSV) is a major health problem in young cattle and accounts for important losses in the industry. Maternal immunity to BRSV is often insufficient to protect against field virus infection, but might still interfere with active immunization. In order to provide efficacious protection from a very young age it is essential that vaccines are able to break through maternal immunity and confer protection shortly after vaccination. The basic vaccination schedule for most inactivated BRSV vaccines consist of two applications with an interval of 3 to 4 weeks. In this study, we determined the immunogenicity of a commercially available inactivated BRSV-Parainfluenza type 3 - Mannheimia haemolytica combination vaccine (Bovilis® Bovipast RSP/Bovilis® Bovigrip) after single vaccination. An experimental model with calves at two weeks of age with moderate to high levels of maternal antibody was used. Five calves were vaccinated and challenged together with five age matched control calves 4 weeks later. The animals were observed for clinical signs

typical for BRSV infection. The challenge virus excretion with nasal discharge was measured. Parameters of the humoral (neutralising and ELISA antibody) and cellular immune response (lymphocyte stimulation assay) were determined. The calves were killed 7 days after challenge for post mortem examination. The clinical signs after challenge were rather mild, probably due to the residual maternal antibody. As expected the single vaccination, did not have any effect on the decline of BRSV-specific neutralising or ELISA antibody. However, the cellular immune system was primed by the vaccination. On group level, virus excretion with nasal discharge was reduced and less virus could be re-isolated lung tissues in the vaccinated group. Moreover, the lungs were less affected by the challenge virus infection in the vaccinated animals.

From these results, it can be concluded, that a single vaccination with an inactivated BRSV vaccine was able to break through the maternal immunity and induce partial protection in very young calves.

Field efficacy study to compare two combination vaccines against major bovine respiratory pathogens in calves

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The bovine respiratory disease complex (BRDC) is a single clinical entity of bronchopneumonia, but it is recognized as a multi factorial disease. There are a number of viruses and bacteria that have been consistently identified as being involved in the disease: bovine respiratory syncytial virus (BRSV), bovine herpesvirus (BHV 1), bovine parainfluenza type 3 virus (PI3), *Mannheimia haemolytica* (Mh) and *Pasteurella multocida* (Pm). Therefore, combination vaccines are widely used to control the BRDC.

The objective of this study was to compare the efficacy of two commercially available combination vaccines in calves under field conditions.

The trial was performed according to a randomized partially blinded controlled study design in France. Fourteen cow-calf farms with a history of BRDC have been selected for the trial. In total, 719 animals were admitted. At admission, one group was vaccinated with an inactivated BRSV-PI3-Mh combination vaccine (Bovilis® Bovipast RSP-Bovilis® Bovigrip) and the second group with a vaccine consisting of attenuated BRSV and PI3 together

with inactivated adjuvanted BVDV. The vaccinations were repeated 4 weeks later. A third group of animals were left unvaccinated to serve as negative controls.

The efficacy of the vaccines was judged by the following parameters: i) number of treated animals because of respiratory disease, ii) number of individual antibiotic treatments per calf for respiratory disease and iii) mortality (death and culled) due to respiratory disease. Animals were monitored for a period of about 4 months after vaccination.

In this study, the incidence of BRDC was unexpectedly low during the observation period on all fourteen farms. However, the results indicate that the vaccinated animals required less antibiotic treatment and less mortality was recorded after vaccination.

The group vaccinated with Bovilis® Bovipast RSP performed significantly better than control group with regard to treatment rate and mortality. In contrast, vaccination with the second product did not result in a significant difference from the control group for these parameters.

Compatibility of a live IBR marker vaccine with an inactivated BRSV-PI3-*Mannheimia haemolytica* combination vaccine as determined by challenges

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Deletion of glycoprotein E (gE) of the bovine herpesvirus type 1 (BHV 1) has been shown to attenuate the virus, making the respective gE deletion mutants suitable as live vaccine (IBR marker vaccines). However, little is known, as to whether these strains cause immunosuppression like the wild type viruses. We investigated whether a live IBR marker vaccine (Bovilis® IBR Marker) hampers the efficacy of an inactivated BRSV-PI3-*Mannheimia haemolytica* (*Mh*) combination vaccine Bovilis® Bovipast RSP, when both products are applied simultaneously.

The efficacy against BRSV, PI3 and *Mh* was determined in two challenge studies in two-week old colostrum deprived calves. In the first study, one group of calves was vaccinated both, intranasally (IN) and intramuscularly (IM) with Bovilis® IBR Marker vaccine and simultaneously subcutaneously (SC) with Bovilis® Bovipast RSP. As a positive control, a second group was only vaccinated with the inactivated vaccine. Both groups of animals were re-vaccinated with Bovilis® Bovipast RSP after four weeks and challenged three weeks later with BRSV together with a third group. Five weeks later, all three groups of animals were challenged with PI3 virus.

In a second study, groups 1 and 2 received Bovilis® IBR marker either IM or IN at the same time as the first dose

of Bovilis® Bovipast. Group 3 was only given the two doses of Bovilis® Bovipast and a fourth group was not vaccinated. All animals were challenged with *Mh* two weeks after the second vaccination. The animals were euthanized between one and three days after challenge. Lung samples were processed for isolation of *Mh*.

In both studies, clinical observations were recorded. Nasal swab samples were tested for challenge virus/bacteria excretion and the development of antibody was followed.

Both vaccination regimes (single and combined vaccine) produced evidence of protection against BRSV and PI3 demonstrated by shorter duration of virus shedding, delayed onset, fewer numbers of animals shedding virus and lower virus titres being recovered.

Likewise, concurrent administration of both vaccines resulted in good protection against *Mh* challenge in terms of reduction of clinical signs, lung lesions and lower levels of *Mh* re-isolation from the lungs. Antibody responses to *Mh* were consistent between all three vaccinated groups. In conclusion, the Bovilis® IBR Marker vaccine was found not to interfere with the efficacy of the different components of Bovilis® Bovipast RSP when administered simultaneously.

Protection of calves against experimental challenge with salmonella dublin and *Salmonella Typhimurium*

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Salmonellosis has been recognized as a disease in cattle all over the world for more than two decades. It has primarily been associated with *S. enterica* subsp. *enterica* serovar Dublin (*S. Dublin*) and *S. enterica* subsp. *enterica* serovar Typhimurium (*S. Typhimurium*). In view of the economic importance to the dairy and calf rearing industries and the potential to infect the human population, different vaccines have been developed.

Two challenge studies with a similar experimental design were performed in calves aged 3-4 weeks: One group of calves was vaccinated twice with the inactivated vaccine Bovivac® S (Intervet international bv) according to the product data sheet. Bovivac® S contains inactivated cells of *S. Dublin* and *S. Typhimurium*. A second age matched group was left unvaccinated and served as control. Two or three weeks after the second vaccination, calves were challenged with either live, virulent *S. Dublin* or *S. Typhimurium*. The animals were monitored for two weeks for clinical signs and samples were collected to determine shedding of the challenge organisms.

After challenge with *S. Dublin*, control calves rapidly succumbed to a severe and overwhelming salmonellosis with 50% of animals being euthanized on human grounds within eight days after challenge. In contrast, disease

was significantly reduced in the vaccinated group and all the calves survived to the end of the study. Moreover the shedding of the challenge organism was greatly reduced in the vaccinated group.

The challenge with *S. Typhimurium* was less severe. 50% (3/6) of the control calves developed a moderately severe salmonellosis characterized mainly by the presence of loose mucoïd diarrhoea and persistent shedding of high numbers of salmonellae. In contrast, all vaccinated calves (n=7) displayed only very mild symptoms of disease with a significant reduction in the magnitude and duration of pyrexia. The shedding of the challenge organisms was also markedly reduced in the vaccinated group. Re-isolation of the challenge strain was achieved from post mortem mesenteric lymph node tissue in five out of six control calves compared to just one out of seven of vaccinated animals.

These data demonstrate the efficacy of Bovivac® S to diminish the severity and duration of clinical disease following *S. Dublin* and *S. Typhimurium* infection. In addition, the vaccine has also demonstrated to reduce faecal shedding of the two organisms and therefore controlling the contamination of the environment.

A follow up serological study on *Neospora caninum* infection in herds affected by abortion in Italy

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Bovine neosporosis is a worldwide parasitic disease that is considered as one of the most important cause of abortion in cattle. Serological investigations have shown that *Neospora caninum* (*N. caninum*) is widespread among Italian cattle, even sometimes in the absence of abortion and without known contacts to dogs. In order to update the information on the seroprevalence of *N. caninum* infection in dairy cattle in Italy, a retrospective serological study has been performed. The study was carried out on 5,564 dairy cattle, from 522 Italian dairy farms that experienced abortion outbreaks between January 2002 and August 2005. Out of all the samples 264 were collected from cows that had aborted. Sera were tested for *N. caninum* antibodies by indirect immunofluorescence test (IFAT). A serum showing an antibody titre $\geq 1:300$ was considered positive. The overall antibody prevalence was 24.8% for serum samples and 74.1% for the farms; 45.5% of the aborting cows were found serologically positive. The percentage of the positive animals showed a decrease during the years: 30.5% in 2002, 27.3% in 2003, 23.7% in 2004 and 16.8% in 2005. The same trend was observed

in the seroprevalence of the aborting cows: 56.6% of the animals were found positive in 2002, 38.7% in 2003, 36.2% in 2004 and 29% in 2005. Herd prevalence remained stable during this period (75.3% in 2002, 78.2% in 2003, 75% in 2004 and 68% in 2005). The trend in within-herd prevalence is comparable with data obtained during a 4-year period in 5 farms that implemented some preventive measures namely culling of cows with repeated abortion: 68% in 2002, 65% in 2003, 55% in 2004 and 27% in 2005. The main transmission route is in utero from cow to calf and at present there are no vaccines against *N. caninum* available in Europe. Management measures such as the identification and culling of repeated aborting cows and the insemination of infected heifers/cows with beef cattle semen seems to be a possible way to reduce the damage caused by the infection. In conclusion, the data obtained in this study confirm that *N. caninum* infection is widespread in the Italian dairy herds and suggest the need for effective tools to prevent the damage caused by the disease in breeding cattle herds.

Bovine neosporosis and the incidence of abortions in a dairy herd

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Neosporosis is a disease caused by the protozoan *Neospora caninum*. In 1989 the first case of neosporosis was confirmed in cattle. Since then several studies have demonstrated it as one of the major causes of abortion in cattle worldwide. In Brazil neosporosis has been identified in dogs and bovines in many States.

In 2000 *N. caninum* was diagnosed via serological (ELISA) and parasitological (isolation and PCR) tests in a dairy herd with a history of endemic abortions in the State of Parana (Brazil).

The prevalence of sero-positive animals for *N. caninum* was 34.8% (60/172). The abortion rate for the sero-positive cows was 20.3% (36 abortions in 177 gestations) and for the sero-negative cows was 6% (21/350). Some calves from sero-positives cows were tested pre-colostrum and were positives in the ELISA test, confirming the vertical transmission of *N. caninum*.

N. caninum (strain BNC/PR3) was isolated from an aborted fetus (7th month of gestation) confirming the abortion due to neosporosis. Control measures were implemented to reduce the problem: dogs were kept closed and did not have access to the feed and drink water of the cows, placenta and aborted fetuses or carcasses; some positive cows that aborted more than two times were culled.

In 2004, a new investigation was carried out in the herd. Serum from 163 animals was collected and analyzed by ELISA. The prevalence of positive cows was 24% (40/163). The abortion rate for the sero-positive cows was 17.9% (10/56) and for the sero-negative cows was 2.7% (4/147). The decrease in the prevalence (34.8 to 24%) is believed to be due to

- efficacy of vertical transmission is less than 100 %.
- That was proven because some daughters of positive cows, that were born after 2000, were sero-negatives - 21.7% (5/23);
- culling of sero-positives cows which aborted more than two times;
- control of horizontal transmission (dogs);
- positive cows were less efficient in their reproductive performance (more abortions), leaving less descendents in the herd.

Since 2004 this herd is being vaccinated against neosporosis. It is expected that the abortion rate in the positives cows will go down.

It can be concluded that the main cause of abortion in this herd was neosporosis and that the implementation of control measures contribute to reduce the impact of the disease.

Year	Prevalance of PositiveCows	Abortion Rate (abortion/gestations)		
		Herd Avg.	Pos. Cows	Neg. Cows
2000	34.8% (60/172)	10.9% (57/522)	20.3% (36/172)	6% (21/350)
2004	24.5% (40/163)	6.9% (14/203)	17.9% (10/56)	2.7% (4/147)

Protection against abortion caused by IBR in pregnant heifers from a single subcutaneous vaccination with a modified live, multivalent vaccine at pre-breeding

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The infectious bovine rhinotracheitis (IBR) virus has long been recognized as a significant cause of abortion in cattle. In order to reduce or eliminate this costly infectious disease, vaccination of females to prevent infection and subsequent abortion is necessary. The objective of this study was to determine the efficacy of a single, pre-breeding vaccination with Vista™ 5 SQ for the prevention of IBR abortion in previously naive heifers challenged with IBR at 170-180 days in gestation.

Sixty healthy, open heifers, seronegative to IBR were included in the study. Heifers were randomized to two treatments: Group 1 heifers were vaccinated with a single 2 ml, subcutaneous minimal protective dose of Vista 5 SQ (n=30) and Group 2 heifers (controls) remained unvaccinated (n=30). Vista 5 SQ vaccination was administered 37 days pre-breeding. Cell-mediated immunity (CMI) was evaluated four months post-vaccination. All pregnant heifers (vaccinated n=16, controls n=14) were challenged at 170-180 days in

gestation with Cooper strain IBR virus. Heifers were evaluated during the post-challenge period for rectal temperatures, clinical scores, and viral shedding from the nasal cavity and vagina. Four months post-vaccination Vista 5 SQ heifers had a greater ($p>0.05$) CMI response than controls. Vista 5 SQ demonstrated a greater antibody response 14 days post challenge, which is consistent with an anamnestic response to the IBR virus in previously vaccinated animals. One hundred percent (100%) of control heifers aborted their fetuses. Meanwhile, 62.5% of Vista 5 SQ vaccinated heifers were protected ($p<0.001$) from IBR abortion. Consistent with previous studies with Vista 5SQ that demonstrated efficacy and a duration of immunity for at least 182 days for IBR, Vista 5 SQ reduced rectal temperatures, clinical scores, nasal and vaginal virus discharge and abortion following challenge when compared to control heifers. Therefore Vista 5 SQ was shown to be efficacious as an aid in reduction of abortions caused by IBR.

Protection against fetal persistent infection (PI) caused by BVDV type 1 and BVDV type 2 for a single vaccination with a multivalent modified live vaccine pre-breeding

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Persistently infected (PI) animals born with either bovine viral diarrhoea (BVD) type 1 or type 2 are considered the major source of infection for naïve contact animals. Prevention of fetal infection is essential for control of BVD. The objective of these studies was to determine the efficacy of a single pre-breeding vaccination with a new multivalent modified live vaccine for the prevention of PI fetuses when pregnant heifers were challenged at 75-84 days of gestation with BVD type 1 and type 2 viruses.

Open, healthy heifers seronegative to both BVD type 1 and type 2 (n=150) were vaccinated pre-breeding with a single minimum protective dose (MPD) of Vista™ 5 SQ to determine the efficacy of preventing fetal persistent infection caused by BVD type 1 and type 2. Heifers were randomized to two treatment groups: (1) the first group was vaccinated subcutaneously with a 2 ml, single MPD of Vista 5 SQ at four weeks pre-breeding; and (2) a second group consisted of controls receiving a placebo vaccination with sterile diluent. There were 68 heifers pregnant after breeding: 23 Vista 5SQ BVD type 1-challenged heifers, 22 Vista 5 SQ BVD type 2-challenged heifers, 12 control BVD type 1-challenged heifers and 11 control BVD type 2-challenged heifers.

Vista 5 SQ vaccinated heifers developed specific serum neutralization (SN) antibody to BVD type 1 and type 2 with high titres following vaccination. All heifers were challenged at approximately 75 days in gestation with either type 1 or type 2 non-cytopathic BVD strains obtained from aborted fetuses (SDS Univ.). The heifers were evaluated in a two-week post challenge period for rectal temperatures, clinical scores, white blood cell counts, viremia and viral shedding from the nasal cavity. All control heifers developed leucopenia (white blood cell decrease), clinical sickness and viremia after challenge, but Vista 5 SQ vaccinated heifers were protected from the challenge. Fetuses were collected at about 150 days of gestation from heifers, and tissue samples were taken for BVD virus isolation. Results demonstrated that 100% of fetuses from unvaccinated heifers were persistently infected following BVD type 1 or type 2 challenges. Vaccination with Vista 5 SQ provided 96% and 91% protection ($p < 0.01$) from fetal persistent infection for BVD type 1 type 2, respectively. These data demonstrate that the vaccine is efficacious as an aid in the prevention of persistently infected calves caused by BVD type 1 and type 2.

Duration of immunity of the IBR fraction of a new modified-live combination vaccine against respiratory disease

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The infectious bovine rhinotracheitis (IBR) virus is ubiquitous in cattle populations around the world. Although it is uncommon to diagnose “classical” IBR disease syndromes in modern production systems, IBR virus is still isolated and associated with disease outbreaks in numerous cattle production systems of all kinds. Vaccination is commonly applied to control the virus spread within and between herds. A duration of immunity (DOI) of 6 months would coincide well with a number of production parameters for both beef and dairy. The potential suppression of disease or repression of recrudescence over an extended period lends itself to those situations where revaccination is not possible or desirable. The objective of this study was to determine the duration of immunity of the IBR fraction in a new Bovine Rhinotracheitis-Virus Diarrhea (Type I and II)-Parainfluenza 3-Respiratory Syncytial Virus modified live vaccine (Vista 5 SQ) in naïve animals.

Thirty one calves, 8 months of age and seronegative to IBR were included in the study. They were randomized to two treatments. Treatments were (1) vaccinated (n=21) with a single 2ml, subcutaneous Minimum Protective Dose (MPD) of Vista 5 SQ or (2) nonvaccinated (n=10). One hundred and

eighty days following a single vaccination all calves were intranasally challenged with a virulent strain of Cooper IBR. Cattle were observed for rectal temperature, clinical signs of disease and virus shedding. Eighty one percent of the vaccinated calves had IBR specific antibodies the day of challenge (182 post vaccination) and exhibited a strong anamnestic response following challenge. None of the control calves developed IBR SN titers up to 182 days. The proportion of calves with clinical signs and the mean number of days with clinical signs was higher ($P<.05$) for controls compared to Vista 5 SQ vaccinated calves. Control calves had higher ($P<.05$) rectal temperatures on days 4 through 9 post challenge compared to those vaccinated with Vista 5 SQ. Control calves shed more ($P<.05$) virus on day 4 and days 7 through 10 post challenge compared to calves vaccinated with Vista 5 SQ. The proportion of control calves shedding virus was more ($P<.01$) than in vaccinates post challenge. The IBR fraction of Vista 5 SQ provided clinical protection from signs of IBR infection, demonstrated immunological response and reduction of time and amount of shedding following challenge 6 months (182 days) from vaccination of naïve animals.

Duration of immunity of the BVDV fractions of a new modified-live combination vaccine against respiratory disease

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Bovine Viral Diarrhoea (BVD) is purported to be the most costly infectious disease to both beef and dairy operations. Vaccination has proven to be an important adjunct control mechanism in conjunction with proper management to restrain the virus spread within and between herds. A duration of immunity (DOI) of 6 months would coincide well with a number of production parameters for both beef and dairy. The objective of the studies reported here was to determine the duration of immunity of the BVD fractions in a new Bovine Rhinotracheitis-Virus Diarrhea (Type I and II)-Parainfluenza 3-Respiratory Syncytial Virus modified live vaccine (Vista™ 5 SQ) in naïve animals.

Two trials - one for each BVD types 1 and 2 were performed including a total of 63 calves, at 5-8 months of age, all seronegative to both BVD types 1 and 2 prior to the studies.

Treatments were: (1) vaccinated (n=20 BVD type 1, n=22 BVD type 2) with a single 2 ml subcutaneous minimal protective dose of Vista 5 SQ or (2) non-vaccinated (n=10 BVD type 1, n=11 BVD type 2). Six months after vaccination, all calves in each trial were challenged with virulent BVD

type 1 or type 2 respectively. Virus neutralizing antibody titres were determined. Cattle were observed for rectal temperatures and clinical signs of disease associated with BVD. Nasal swabs were tested for challenge virus excretion.

All vaccinated calves developed specific BVD type 1 and type 2 neutralizing antibody, which persisted until the time of challenge, whereas the control animals remained seronegative during the same period.

In both studies, clinical scores were higher ($p < 0.05$) for non-vaccinated calves. In the BVD type 2 challenge study, 45% of the negative control calves died whereas all vaccinated calves survived the challenge. Vaccinated calves did not develop ($p < 0.05$) leucopenia or thrombocytopenia when compared to controls post challenge with BVD type 1 or 2. In both trials, vaccinated calves had less ($p < 0.05$) virus isolated from the nasal cavities after challenge.

From these results, it can be concluded that the BVD fractions of the vaccine aided in the control of disease caused by BVD types 1 and 2 and reduced the virus excretion as long as 6 months after vaccination.

Efficacy of a single dose of multivalent *Leptospira-Campylobacter* vaccine against virulent challenge

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Leptospirosis and bovine venereal campylobacteriosis are commonly known as economically important bacterial infection of livestock that affect negatively reproductive performance. The efficacy of a single 2 ml subcutaneous dose of a multivalent bacterin (Vista™ VL5 SQ) containing *campylobacter fetus-Leptospira canicola-grippotyphosa-hardjo-icterohaemorrhagiae-pomona* in a proprietary new adjuvant following virulent challenge was determined.

Six studies (one for each antigen) were conducted. The bacterin was formulated to contain the minimum protective antigen level for each fraction tested. Efficacy was determined by *leptospira* isolation from blood and urine and by pregnancy rates for *Campylobacter fetus* following a virulent challenge 21 days post-vaccination, at which time the bulls were introduced into the herds.

There were a total of 187 cattle included in the six studies. Cattle were at two to eight months of age for the *Leptospira* challenge and 8 to 12 months of age for the *Campylobacter fetus* challenge. Cattle vaccinated with VL5 SQ had an increase ($p<0.006$) in serological response at 21 days post vaccination for all *Leptospira* antigens. There was a reduction ($p<0.008$) in the number

of animals having *Leptospira canicola*, *grippotyphosa*, *icterohaemorrhagia* and *pomona* organisms isolated when compared to non-vaccinates. Vaccination of cattle with *L. canicola* and *L. pomona* fractions prevented 100% of vaccinated animals from having any *L. canicola* or *L. pomona* organisms isolated in the blood. For the *L. hardjo* study, cattle were challenged with a virulent *borgpetersenii hardjo (hardjo-bovis)* strain 203. VL5 SQ prevented ($p=0.005$) isolation of leptospires from the urine in all vaccinated cattle (0/15) while leptospires were isolated from 87% (13/15) of the non-vaccinated cattle. VL5 SQ vaccinated cattle had an improvement ($p=0.02$) in pregnancy rates compared to non-vaccinates (68% vs 41%) following a *Campylobacter fetus* challenge.

These data demonstrate that a single 2 ml subcutaneous dose of VL5 SQ is efficacious as an aid in reducing infertility (reproductive disease caused by *Campylobacter fetus*) and as an aid in preventing leptospirosis (caused by *Leptospira canicola*, *L. grippotyphosa*, *L. hardjo* - including the *L. borgpetersenii* serovar *hardjo bovis*, *L. icterohaemorrhagiae* and *L. pomona*). VL5 SQ prevents urine shedding following challenge with *borgpetersenii hardjo (hardjo-bovis)* in 100% of the vaccinated animals.

Vaccination with a live 5-way combination vaccine does not have a negative effect on milk yields

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Maintenance of milk yield is a major concern when it comes to vaccination of dairy herds, especially. The effect of a new biological product on this parameter can not be predicted but should be determined by controlled field trials.

A study was performed to determine the milk production after vaccination with a new, adjuvanted, 5-way combination vaccine (Vista™ 5L5 SQ) containing attenuated Infectious Bovine Rhinotracheitis (IBR) Virus, Bovine Respiratory Syncytial Virus (BRSV), Bovine Parainfluenza Type 3 Virus (PI3), Bovine Viral Diarrhoea Virus (BVDV) Types 1 and 2 and 5 different serotypes of *Leptospira*.

176 milking cows were included in the study. One group of 97 animals was vaccinated subcutaneously with 2 ml of

the vaccine according to the data sheet recommendations. A second group of 79 animals was administered similarly 2 mls of a saline solution to serve as negative control. Mean lactation for the Vista™ 5L5 SQ was 2.4 while it was 2.7 for the negative control group. Milk production was measured for 11 days prior to the vaccination to establish baseline values and to stratify the cows and assign them to one of the two treatment groups. Milk production was measured during 7 days post-vaccination.

There were no differences ($p > 0.05$) in milk production between the two treatment groups.

Therefore, it can be concluded, that Vista™ 5L5 SQ can be used in milking cows without any negative effect on production.

AbstractBook

