IntraNasal BRSV and PI3 vaccination:
Innovation for the prevention of respiratory disease in cattle
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A new vaccine for BRSV and PI3 protection: Rispoval IntraNasal

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Bovine respiratory disease

Infectious respiratory disease in calves and young cattle is a source of considerable loss to the cattle industry worldwide. It is a significant problem as calves and fattening cattle are housed for considerable periods of time. It presents a complex problem because the disease syndrome is influenced by factors of management and stress and because it involves both viral and bacterial infection. In many intensive and semi-intensive systems of cattle management, the prevention of respiratory disease is associated principally with the use of vaccines, formulated to protect against the major viral agents responsible for bovine pneumonia.

In young calves the most important viruses are PI3V and BRSV. BRSV is recognised as a particularly virulent pathogen, capable of causing severe acute respiratory disease with death in some cases, whereas PI3V tends to be regarded more as a common pre-cursor to bacterial infection rather than a major pathogen in its own right. Vaccination of young calves against these two viruses is essential on many farms in order to reduce the impact of the viral infection and any secondary potential bacterial infection, reducing losses from severe acute disease and the long-term welfare and economic implications of chronic respiratory disease. The use of antiviral therapy is not economically feasible in cattle. There are two situations in which respiratory disease in cattle is common and PI3 virus and BRSV are frequently implicated. In both situations calves are assembled, often from different sources, and mixed, either as young calves for rearing, when disease often occurs from about one month of age, or in recently purchased, previously single suckled calves aged from four to eight months of age, weaned from the beef herd. However, calves from a single source, including home-bred animals, can also be affected where carrier animals (usually the adult cattle) are present in the herd or a single infected animal is imported into the herd. In some dairy herds calves may be infected at a very young age. For both BRSV and PI3 maternally derived antibody (MDA), although nearly always present, provides poor protection from clinical disease. For other viral respiratory pathogens, such as BVD and BHV1 (IBR) MDA is more often effective.
Vaccines administered by the intranasal route have been available for several years for Pi3 virus and have been shown to be effective. BRSV vaccines have only been available for administration by the intramuscular or subcutaneous route and some vaccines (using formalin killed virus) were shown to enhance the pathogenicity of BRSV when calves were subsequently challenged. This adverse reaction to infection following vaccination was also seen in human children with Respiratory Syncytial Virus. More recent intramuscular vaccines (using modified-live virus) have been free from side effects but have required repeat vaccinations if the first dose was given whilst MDA was still present in the calf. The development of an intranasally administered BRSV vaccine, which can induce early immunity in the presence of MDA, using a single dose at three weeks of age, will be a major advance in protecting very young calves.

The viral strains (Pi3V and BRSV) used in the production of the vaccine (Rispoval™ RS+Pi3 IntraNasal) reviewed in this paper are the same as those in the vaccines Rispoval™ 4 and Rispoval™ 3 which are currently available for intramuscular use in cattle. These vaccines have been used commercially for several years and have an excellent safety record.

Rispoval™ RS+Pi3 IntraNasal

Rispoval RS + Pi3 IntraNasal is a bivalent vaccine containing two modified live viruses (Bovine Parainfluenza, type 3 virus (Pi3) ts strain 103 and Bovine Respiratory Syncytial Virus (BRSV) strain 375) in a freeze-dried fraction.

The modified live fraction is reconstituted with 2mL of liquid fraction (saline) before intranasal administration to cattle. Rispoval RS + Pi3 IntraNasal is indicated from 3 weeks of age against BRSV and Pi3, to reduce the duration and titre of viral shedding associated with BRSV and Pi3 viruses.

A single dose ensures protection for at least 9 weeks following vaccination as demonstrated by challenge in animals seropositive at time of administration. In seronegative animals, a significant reduction in BRSV shedding has been demonstrated in animals challenged as early as 5 days after vaccination. Protection against both viruses is achieved by 10 days of vaccination as demonstrated by challenge. Animals vaccinated intranasally at 3 weeks of age can be revaccinated with an appropriate intramuscular vaccine at approximately 3 months of age to achieve continuing protection, perhaps with the inclusion of additional antigens.

History of Pfizer Animal Health’s BRSV and Pi3V combination vaccines

In the mid-’90s, PAH developed and registered respiratory vaccines, which protected cattle against diseases produced by BRSV, Pi3, BVDV and BHV-1 (IBR). These vaccines were registered in several EU Member States under the names of Rispoval 4 and CattleMaster 4. An additional vaccine containing BRSV, Pi3 and BVDV (Rispoval 3) was registered in 18 European countries by Mutual Recognition in February 2005. Although these products can provide cattle with broad protection against the respiratory viral pathogens, it was recognized that there was still an unmet need for a vaccine to protect the very young calf, particularly from BRSV infection.

Providing protection at a young age presents several technical challenges. Maternal immunity is generally present so any vaccine must be able to act in the presence of maternally derived antibodies. More importantly, a way must also be found to shorten the traditional timeline from first vaccination to the onset of immunity, where protection is typically established only some time after the second of two vaccine doses. The intra-nasal route potentially offers a solution to both these difficulties and Rispoval IntraNasal has been developed to take advantage of this, using two well characterised and proven viral strains from within the Pfizer range.

Is the vaccine safe?

One of the main issues for a modified live vaccine given intranasally is its safety in the vaccinated animal and for in contact animals. All studies for Rispoval RS + Pi3 IntraNasal were carried out with the final formulation. The trials were designed to fulfill European Directive EC/2001/82 as well as relevant European Directives, Guidelines and European Pharmacopoeia monographs in force. Most of the studies were carried out according to the principles of GLP (Good Laboratory Practice). Laboratory safety trials were performed with the vaccine reconstituted at maximum release titres.

Two studies were carried out to address the spread and dissemination of the vaccine strains. As expected for a vaccine administered intranasally, vaccinated animals were shown to shed vaccine viruses by collection of nasopharyngeal swabs. In addition seroconversion was observed in seronegative control in-contact animals sharing the same airspace as the vaccinates. Evidence for a lack of dissemination within the tissues of vaccinated animals was also seen in human children with Respiratory Syncytial Virus. More recent intramuscular vaccines (using modified-live virus) have been free from side effects but have required repeat vaccinations if the first dose was given whilst MDA was still present in the calf.

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calves was shown following intramuscular injection of the freeze-dried fraction of Rispoval RS+Pi3 IntraNasal. The two attenuated strains could not be isolated in any of the tissues observed four days after an overdose administration of the vaccine. Intramuscular administration being far more invasive than intranasal application, this study contributes to demonstrate the absence of spread and dissemination of the two attenuated strains.

As the vaccine strains were shown to spread to susceptible in contact calves it was important to demonstrate that this did not represent a health risk. Two separate studies were carried out to verify the absence of reversion to virulence for the two vaccinal strains. Both studies were carried out on minimum age animals in accordance with the requirements given in the appropriate European Pharmacopoeia monographs. The titres of the first administration of intranasal inoculum were maximal and the passage levels used were as close as possible from the Master Seed to increase the chance of observing a reversion to virulence using the least attenuated passage. No adverse reactions were found during either study.

To comply with Regulatory requirements the safety of the administration of one dose, an overdose and the repeated administration of one dose was examined in seronegative three week-old calves. Two ten-fold overdose studies were performed where calves received intranasally 10 times the amount of BRSV and Pi3 that could be at maximum present in the commercial formulation. All administrations of the vaccine were carried out intranasally according to the recommended instructions on the SPC. A transient increase in rectal temperature was recorded in the ten-fold overdose vaccinated animals.

In a second ten-fold overdose study the vaccine was intranasally administered to 8 three week old animals and 7 one week old animals. Another 4 three week old animals were kept as untreated controls. The study was carried out according to GLP. No signs of respiratory disease were observed in any of the animals within 21 days after the overdose administration. No rise in rectal temperatures was observed within seven days after the overdose administration. Results are fully compliant with the Ph. Eur. Monographs and confirmed the safety of an overdose in three week old animals. The second study also confirms safety of a vaccine overdose in one week old animals.

The vaccine has also been shown to be safe in three GCP(V) compliant field trials carried out in UK in minimum age animals. These studies combined safety and efficacy observations in animals of dairy and beef breeds. In one of these studies animals were vaccinated twice specifically to determine safety upon re-exposure to BRSV. No adverse reactions, including hypersensitivity, were observed during the studies.

Therefore Rispoval RS + Pi3 IntraNasal is safe when administered to calves 3 weeks old or above, as an overdose or a repeated dose. The other aspects of the safety requested by the European Directives and Guidelines have also been successfully demonstrated. The strains of viruses used to produce Rispoval RS + Pi3 IntraNasal have been used for several years to produce other vaccines, all of which have proven safety records. The safety profile of the Rispoval RS + Pi3 IntraNasal formulation was therefore not unexpected.

Does it work?

A calf with typical signs of respiratory disease caused by BRSV infection

All efficacy studies for Rispoval RS + Pi3 IntraNasal were carried out with the final formulation, as used for the commercial product. Efficacy studies were conducted with vaccine at titres below minimum release levels. Efficacy was therefore demonstrated using potentially a slightly less potent vaccine than would be available commercially. The trials were designed to fulfil European Directive EC/2001/82 as well as relevant European Directives, Guidelines and European Pharmacopoeia monographs in force for live BRSV and Pi3V vaccines. Laboratory efficacy trials were performed with the vaccine reconstituted according to the SPC recommendations.

To demonstrate the onset of immunity to Pi3V, seronegative (or low seropositive) 16 -26 day-old animals were vaccinated with a single intranasal application of the vaccine. A group of 5 vaccinated and 6 control animals was challenged ten days later with J121 (UK) strain, whilst another group of 6 vaccinates and 6 controls was challenged 21 days later as required by the Ph. Eur. Monograph on live Pi3V vaccines. The titres of the vaccine strains were 4.8 log10 CCID50/dose for BRSV and 4.7 log10 CCID50/dose for Pi3 which therefore constitute the minimum immunising titres.

All of the six vaccinated calves challenged at 21 days post-vaccination showed a serological response to
vaccination at the time of challenge. Vaccinated calves had a faster and stronger response after challenge which was significantly higher than that in the control (unvaccinated) group. The 10 day challenge vaccinated group did not show any serological response to vaccination until the day of challenge.

Compared to non-vaccinated controls, there was a statistically significant reduction of viral shedding (duration and amount of viruses) in the animals challenged even at 10 days after vaccination. The study complies with the requirements for potency in Ph. Eur. Monograph on PI3 vaccines.

Clinical signs after challenge were mild in all the treatment groups and there were no significant differences between any of the vaccinated and control groups. Fever tended to occur in more calves and persist for longer in unvaccinated calves.

To determine the onset of immunity to BRSV seronegative, colostrum deprived 19 to 26 days old animals were vaccinated according to the SPC recommendation with minimum titre vaccine. Three sets of vaccinated and control animals were challenged intranasally at three different times after vaccination (5 days, 10 days and 21 days) using the Odijk (The Netherlands) strain. In the vaccinated 21 day challenge group 66.7% (4/6) of calves showed a serological response to vaccination by the day of challenge (LS mean titres 2.5 to 5.0 log2). No significant serological response to vaccination was seen in vaccinated calves prior to challenge in the 10 and five day challenge groups. Following challenge there was a significant difference in serological titres to BRSV between the vaccinated and control calves in the 21 day challenge groups, the response was faster and stronger in the vaccinated calves; there were no significant differences in serological response between the vaccinated and control calves in the 10 and five day challenge groups. All calves in the 10 day challenge groups had seroconverted by 14 days after challenge. One vaccinated calf had responded by 10 days post-vaccination and by 7 days after challenge two vaccinated calves demonstrated an anamnestic response when no seroconversion was seen in the control animals. In the five day challenge group all animals had seroconverted by 14 days after challenge.

Following challenge, clinical signs, classified from mild to severe, were seen in all the treatment groups. A significant reduction in both coughing in the 10 day vaccinated animals and abnormal respiration in the 5 day vaccinated animals was seen compared to control animals. Other clinical signs were not significantly different between vaccinated and control groups.

All the 21 day challenge unvaccinated calves shed BRSV after challenge for up to five days, whereas only two vaccinated calves shed virus for up to three days after challenge and the remaining four calves never shed virus after challenge. All calves in the 10 and 5 day challenge groups (both vaccinates and controls) shed virus after challenge; significant differences in viral shedding were seen between vaccinates and controls in the 10 and 5 day challenge groups. The reduction of viral shedding due to vaccination increased (duration and mean titre) as the interval from vaccination to challenge increased from 5 to 21 days.

Because the Ph Eur Monographs require the use of calves with no antibodies against BRSV to demonstrate immunogenicity of live vaccines and because all animals in the field practically have
maternal antibodies against BRSV and PI3V at three weeks of age, challenge studies were performed to verify if the single administration of the vaccine at minimum age in seropositive animals was sufficient to ensure protection up to the age of 3 months. The same minimum titre vaccine was used in both studies. The titres used were 5.0 log10 CCID50/dose for BRSV and 5.4 log10 CCID50/dose for PI3.

The maternal antibody titres observed in calves on the day of vaccination in both studies are representative of maternal antibody titres against PI3 or BRSV that are observed in calves under field conditions at the time of vaccination. Because calves were vaccinated at three weeks of age, when levels of MDA against PI3 and BRSV are still very high, the vaccine was tested under circumstances whereby a maximum interference with MDA could have occurred and was still shown to be efficacious. The basic design of the studies was identical. At least 10 animals for each of the control and vaccinated group were used with 2 animals acting as sentinels. Sixty-six days after single administration of the vaccine at minimum age, animals were challenged using either the O'dijk strain for BRSV study or J121 strain for PI3 study.

In the PI3V study the challenge was very mild and the clinical signs were not statistically different between the control and vaccinated animals. The daily mean temperature and the total amount of virus shed were significantly lower for the vaccinated compared to the control animals. Overall, the presence of maternally derived antibodies had no effect on the protective immunity.

The BRSV challenge was severe resulting in the withdrawal of 2 control animals and one sentinel (untreated) animal. Overall, the severity of the clinical signs was reduced for the vaccinated compared to the control animals. Vaccinated animals results were also statistically different to the control regarding the total amount of virus shed, the daily viral shedding and the duration of the viral shedding. The presence of MDA had no effect on the protective immunity.

The laboratory data were complemented by two field trials carried out in UK in minimum age animals, using the vaccine produced in the final method of production, reconstituted and used intranasally with an applicator according to the SPC recommendation. These studies combined safety and efficacy observations. Animals of the first study were of different breeds (48 vaccinates and 25 controls). The second study involved beef animals (61 vaccinates and 31 controls). No natural BRSV or PI3 challenges were observed during these studies.

Conclusions

Rispoval RS + PI3 IntraNasal is efficacious when administered to calves 3 weeks old or above. Studies were conducted in compliance with the Ph Eur Monographs on BRSV and PI3 vaccines. This vaccine is intended for use in the first few weeks of cattle life. Its intranasal application as a single dose is likely to be appreciated by users in the field as it simplifies the handling of animals and therefore reduces stress. For animals vaccinated at 3 weeks, a re-vaccination is required at three months of age for continuing protection. At this time, maternally derived antibodies have generally disappeared, giving the opportunity to use vaccines with an extended spectrum.

References