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Systematic review and meta-analysis comparing arrival versus delayed vaccination of high-risk beef cattle with 5-way modified-live viral vaccines against BHV-1, BRSV, PI3, and BVD types 1 and 2

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Abstract

Bovine respiratory disease (BRD) is a major economic and welfare concern of the beef cattle industry. Various approaches to vaccination against the major viral pathogens have been investigated, including timing. It is thought that beef cattle entering feedlots may not be able to adequately respond to vaccination due to the stresses of shipping and processing. A meta-analysis was performed to compare the effectiveness of on-arrival vaccination to vaccination delayed 7 or more days following arrival, using a modified-live viral (MLV) vaccine against bovine herpesvirus-1 (BHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza-3 (PI3), and bovine viral diarrhea types 1 and 2 (BVD 1 and 2), on outcomes of BRD morbidity, retreatment risk, and mortality. Eight studies were identified comparing vaccination timing in feedlot cattle via literature search. Mantel-Haenzsel risk ratios were calculated for each outcome, and Forest plots were constructed. In the studies identified, there was no difference in BRD morbidity risk, retreatment risk, or BRD mortality between calves vaccinated at arrival or delayed. Based on the data from the studies analyzed, it would not appear that there is an advantage or disadvantage in terms or morbidity, retreatment risk, or mortality in delaying vaccination.

Key words: bovine respiratory disease, BRD, vaccination, shipping fever

Résumé

Le complexe respiratoire bovin (CRB) est une préoccupation importante pour le secteur des bovins de boucherie tant du point de vue économique que du bien-être. Diverses approches de vaccination contre les pathogènes viraux les plus importants ont été étudiées incluant le calendrier de vaccination. On pense que les bovins de boucherie à leur entrée dans les parcs d'engraissement ne répondent pas bien à la vaccination en raison du stress associé au transport et au traitement. À l'aide d'une méta-analyse, on a comparé l'efficacité de la vaccination à l'arrivée à la vaccination après un délai de 7 jours avec un vaccin à virus vivants modifiés contre le virus de type 1 de l'herpès bovin, le virus respiratoire syncytial bovin, le virus parainfluenza 3 et le virus des types 1 et 2 de la diarrhée virale bovine sur des mesures de morbidité associée au CRB, sur le risque de retraitement et sur la mortalité. Suite à une recherche de la littérature, on a identifié huit études qui comparaient le calendrier de la vaccination chez des bovins en parc d'engraissement. On a calculé des rapports de risque de Mantel-Haenszel pour chaque résultante qui ont servi pour faire des graphiques en forêt. Dans les études identifiées, il n'y avait pas de différence dans le risque de morbidité associée au CRB, dans le risque de retraitement ou dans la mortalité associée au CRB selon que la vaccination était faite à l'arrivée ou après un délai. Selon les données provenant de ces études, il ne semble pas y avoir d'avantage ou de désavantage à repousser la vaccination tant pour la morbidité, le risque de retraitement que pour la mortalité.

Introduction

Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in North American beef cattle, and thus a major economic and welfare concern of individuals involved in the beef cattle industry.[21,22] This disease complex's pathogenesis is multifactorial, and is most often due to an initial viral insult, followed by a secondary bacterial infection.[18] The most common viruses implicated in BRD are bovine herpesvirus 1 (BHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza 3 (PI3), and bovine viral diarrhea viruses types 1 and 2 (BVD 1 and 2), and it is common practice to administer vaccines against these viruses to cattle entering feedlots and stocker operations within the United States and Canada.[21] However, there have long been concerns that cattle

may not mount an optimal immune response when vaccinated immediately upon arrival.[12,19] It is thought that elevated cortisol levels due to the stress of transport, commingling, and common husbandry procedures such as weaning, dehorning and castration, may result in immunosuppression and reduce the responsiveness of naïve cattle to vaccination.[2,18] For this reason, some have suggested that delaying vaccination until cattle are acclimated to the feedlot setting may be a more appropriate management tool to optimize a protective immune response and improve animal performance.[10,14]

A number of studies have been conducted to evaluate the effects of vaccination timing on morbidity and mortality, but often include other interventions that might confound interpretation of the results.[4,7,8,13,14,15,16,17] Although common practice in the beef cattle industry, these other interventions, such as metaphylaxis, use of *Mannheimia* leukotoxoid vaccines or other treatments, can make objective evaluation of the effects of only vaccination timing difficult. It is therefore our goal to provide a systematic review of these studies, and to extract relevant data to perform a meta-analysis of vaccine timing on BRD morbidity, retreatment risk, and mortality. The decision to include retreatment risk was made because this outcome might be an indicator of disease severity; animals more severely affected by BRD may be less likely to respond to treatment and may thus require additional antimicrobial intervention. However, if different antimicrobials are used between studies for first-line BRD therapy, retreatment risk could actually be more of an evaluation of antimicrobial efficacy than the actual risk of retreatment in response to vaccination timing. Nevertheless, as this is a commonly reported finding in studies, and other non-invasive evaluators of disease severity, such as ultrasound, are not commonly performed, it was decided that this outcome may be the best commonly reported outcome for estimating disease severity. Therefore, the objective of this analysis was to determine if on-arrival or delayed vaccination with a modified-live virus (MLV) vaccine against BHV-1, BRSV, PI3, and BVD types 1 and 2 is more effective for the prevention of BRD in high-risk beef cattle, through evaluation of morbidity, retreatment risk, and mortality outcomes.

Materials and Methods

Literature Search and Inclusion Criteria

A literature search was performed with the following inclusion/exclusion criteria to find studies that compared the efficacy of arrival vaccination to delayed vaccination with a MLV pentavalent vaccine against viral BRD complex pathogens in high-risk beef cattle. Specifically, the vaccines needed to be labeled for the prevention of or the aid in prevention of disease caused by BHV-1, PI3, BRSV, and BVD types 1 and 2. Time points for interventions needed to include vaccination at arrival feedlot processing, and vaccination at a time point greater than 7 days following arrival. Only studies reported in English were considered. Both studies in peer-reviewed journals and extension publications were evaluated. Studies needed to be conducted on high-risk beef cattle and report clinically relevant outcomes: morbidity, mortality, and retreatments, either as total case numbers or as a percentage of a population. Studies reporting only antibody titers were excluded. It was necessary that all disease be naturally occurring. Means of diagnosis of respiratory disease had to be clearly described, and had to have a clear case definition that included clinical signs of BRD such as depression, diminished appetite, increased respiratory rate, cough, nasal or ocular discharge, and increased rectal temperature.

PubMed and CAB were both searched, first using the terms (timing OR arrival OR delay*) AND (BRD OR respiratory+disease OR pneumon*) AND (bovine OR cattle OR calves) AND (vaccin*) on February 26, 2019. PubMed initially yielded 76 publications, while CAB yielded 100. A second literature search was performed with broader search terms, to find studies that may have been missed in the initial search, using (BRD OR respiratory+disease OR pneumon*) AND (IBR OR BHV-1 OR BHV1 OR BRSV OR PI3 OR BVD) AND (bovine OR cattle OR calves) AND (vaccin*). This search yielded 304 publications from PubMed, and 155 from CAB. Because of the large number of publications to be considered, titles of studies clearly indicating the wrong class of cattle (i.e., veal calves, dairy calves, nursing calves, etc.) or for the wrong disease condition were eliminated, as well as studies only evaluating the effectiveness of vaccines in general and not comparing vaccinations administered during the desired timepoints. Studies evaluating only antibody titers or non-pentavalent vaccines were excluded as well. Studies not excluded were identified, and the abstracts read to help categorize for inclusion or exclusion. The same exclusion criteria were applied to the evaluation of the abstracts as to the evaluation of the titles. At this time, 5 papers were identified,[13,14,15,16] but 1 was excluded for not reporting outcomes for the 2 timepoints, only overall morbidity and mortality.[10] A manual search through the references of the 4 remaining studies identified an additional 2 in extension publications;[8,11] 1 was excluded for only evaluating a monovalent BVD vaccine.[11] One additional study in *The Bovine Practitioner* was identified through this reference search; an additional 2 studies were identified from this same journal by manual search.[4,7,17] This yielded a total of 8 studies for further consideration.

Statistical Analysis

After identification of all suitable studies, a Mantel-Haenszel risk ratio was calculated using a random effects model, along with a 95% CI and presented in a Forest plot, for BRD morbidity, retreatment risk, and mortality.[3] Study weight was determined by the sample size. The Cochran Q statistic was also calculated. A $P \geq 0.10$ for this statistic, and an $I^2 > 50\%$ were used to indicate potential heterogeneity of the studies. A confidence interval that crosses 1 was considered indicative of no significant difference between the compared variables.

Study Quality

Quality assessment was performed on the 8 identified studies. These quality factors included random allocation of animals, clear BRD case definition and method of diagnosis, blinding of individuals evaluating the animals for respiratory disease, timing of delayed vaccination, timing of outcome assessment, and other factors, such as metaphylaxis or administration of other respiratory vaccines that may confound the results (Table 1). All studies state that they were randomized, but only 4 studies had a clear description of how randomization was performed. All studies had clear case definitions for BRD diagnosis, however, 2 studies specified a rectal temperature ≥ 103°F (39.5°C) as being indicative of disease, whereas all other studies used a temperature ≥ 104°F (40°C). Only half of the studies had blinding of the individuals assessing animals for illness. All studies clearly defined both time points for arrival and delayed; however, delayed vaccination timing ranged from 7 to 30 days following arrival. The timing of outcome assessment varied a great deal in the studies, and ranged from 28 days after arrival to closeout at 260 days. Two studies assessed outcomes at multiple timepoints: the Hagenmaier study at days 111 and 260, and the Rogers study at days 60, 116, and closeout (196 to 221 days, average was 209 days).[7,17] To make comparisons more relevant to assessing BRD outcomes in the early feeding period, the earliest timepoint was selected from each study (111 and 60 days, respectively). The use of the 111 day timepoint in the Hagenmaier study does have the potential to result in an artificially inflated value for the BRD outcomes assessed purely due to having more time for these events to occur; however, because BRD incidence is known to decrease dramatically once cattle are no longer in the early feeding period, this timepoint was nevertheless included.[5,7]

Many of the studies additionally evaluated other factors that could perhaps serve as confounders to the assessment of vaccination timing (Table 2). There were 2 studies that included the use of *Mannheimia haemolytica* leukotoxoid, or leukotoxoid and outer membrane proteins in both groups at arrival.[7,17] The effects of these products on BRD outcomes have been discussed elsewhere, but are generally found to be positive.[9] However, because the overall risk ratio for morbidity for these and similar products is near 1, and because the treatment was utilized in both groups, it was decided to include them in the analysis, but also present Forest plots excluding these studies.[7,17] One of these studies also included an arm that evaluated an immunostimulant; however, this arm was excluded in evaluation due to the potential effects of the use of this product on outcomes.[17] Another study included an arm evaluating use of an intranasal vaccine against BHV-1 and PI3; this arm of the experiment was also excluded.[4] One study also evaluated the effects of administering clostridial vaccines at arrival and delayed, while another evaluated the effects of timing of growth implant administration at arrival and delayed; however, it was found in those studies that the use of clostridial vaccines and growth implants did not play a role (were not significantly different) in the outcomes of interest (morbidity, retreatment risk, and mortality).[13,16] Thus, the clostridial/implant timing arms were each combined with the arm not utilizing these interventions in their respective studies, such that only timing of MLV vaccination was evaluated. Other than MLV vaccine timing, all calves in all studies were processed at arrival in an equal manner between groups, such as deworming, delousing, castration, and dehorning.

A large proportion of the studies also included boostering of vaccines; 2 boostered only those cattle vaccinated at arrival, such that these cattle received 2 doses of vaccine

Table 1. Summary of evaluated quality indicators in studies.

Study	Randomization	Randomization method described	Blinding of BRD assessors	BRD case definition	Timing of delayed*	Time of outcome assessment*	Metaphylaxis	Leukotoxoid vaccination	Study location	Sample size arrival	Sample size delayed
Duff (2000)	Yes	Yes	No	Yes	7	28	No	No	Small pen	25	25
Hagenmaier (2018)	Yes	Yes	Yes	Yes	28	111	Yes	Yes	Feedlot	855	860
Kreikemeier (1996)	Yes	Yes	No	Yes	21	56	No	No	Small pen	85	88
Poe (2013)	Yes	No	No	Yes	14	42	No	No	Small pen	196	197
Richeson (2008)	Yes	No	Yes	Yes	14	42	No	No	Small pen	264	264
Richeson (2009)	Yes	No	No	Yes	14	56	Yes	No	Small pen	132	132
Richeson (2015)	Yes	No	Yes	Yes	14	42	Yes	No	Small pen	123	123
Rogers (2016)	Yes	Yes	Yes	Yes	30	60	Yes	Yes	Feedlot	1290	1296

*Days following arrival

Table 2. Summary of potential confounders in evaluation of BRD outcomes.

Study	Metaphylaxis		Sick calves at arrival treated		1st antimicrobial treatment		Booster vaccination		Other BRD vaccinations
		PMI*		Excluded		PTI†	Arrival	Delayed	
Duff (2000)					Ceftiofur hydrochloride and penicillin	NS‡	Yes	No	
Hagenmaier (2018)	Tilmicosin	3			Enrofloxacin	3	No	No	*Mh* leukotoxoid
Kreikemeier (1996)					Tilmicosin	2			
Poe (2013)			Yes	NS‡	Florfenicol	3	No	No	
Richeson (2008)			Yes	Yes	Tilmicosin	3	Yes	Yes	
Richeson (2009)	Tilmicosin	2			Florfenicol	2	Yes	Yes	
Richeson (2015)	Tilmicosin	1			Florfenicol	3	Yes	Yes	
Rogers (2016)	Tilmicosin	NS‡			NS‡		Yes	No	*Mh* leukotoxoid

*Post metaphylaxis interval, days
†Post treatment interval, days
‡Not specified

within the study evaluation period (Table 2).[4,17] Three studies utilized boostering of the vaccine in both arrival and delayed groups, and the remaining 3 did not utilize it in either group.[7,8,13,14,15,16]

Studies were additionally checked for the use of additional antimicrobial therapies that may play a role in BRD incidence; it was found that 4 of the studies utilized on-arrival metaphylaxis with tilmicosin.[7,15,16,17] In relation to metaphylaxis and antimicrobial treatments for animals diagnosed with BRD, the post-treatment interval (PTI) or post-metaphylaxis interval (PMI) was additionally evaluated, as this would have an effect on the definition of a treatment failure, and would affect retreatment rate (Table 2). The PTI is the time following treatment with a therapy, in this case an antimicrobial for the treatment of BRD, and the evaluation of that therapy's success or failure.[1] In regards to retreatment risk, if a PTI is too short, an animal could potentially be rediagnosed and retreated for disease, when in fact it is still within the window of therapy for the previous treatment and still healing from the previous "bout," thus, retreatment risk could be artificially increased in this scenario. Three of these studies utilizing tilmicosin metaphylaxis had PMIs ≤ 3 days.[7,15,16] One study did not specify a metaphylaxis PMI.[17] One study also had a treatment arm evaluating the use of chlortetracycline in calves vaccinated at arrival or delayed in addition to the primary arm evaluating timing as the only variable; this arm was excluded from our evaluation, as the method for the CTC feeding was unclear.[8] Another study used tilmicosin on arrival in cattle diagnosed as sick only; however, these animals within the study were excluded from analysis.[14] Another study utilized florfenicol in the same manner, but it is unclear if those calves were excluded from analysis.[13] Three studies utilized florfenicol as the first treatment for animals diagnosed with BRD,[13,15,16] and 2 utilized tilmicosin.[8,14] One study utilized enrofloxacin, and 1 utilized a combination therapy of ceftiofur hydrochloride and pencillin.[4,7] One study did not specify an antimicrobial protocol.[17]

Results and Discussion

Considering the heterogeneity in design of the studies presented, it could be debated that comparison and calculation of an overall risk ratio for each outcome might be inappropriate. Still, it is possible that useful information could be gleaned from a comparison. In regards to morbidity, when all studies are evaluated, the overall risk ratio is 0.99, with a 95% CI that crossed 1 (0.93, 1.06; Figure 1). Heterogeneity (I^2) for this set of studies is 24%, which is below the threshold of 50%, indicating low heterogeneity. Altogether, this would indicate that there is no difference in regard to morbidity between vaccination at either timepoint, and that these studies are not heterogeneous enough in terms of outcomes to preclude analysis. When each study is considered individually, all studies have a 95% CI that crosses 1, although the risk ratios individually are evenly distributed between both sides of the vertical risk ratio bar. When those studies utilizing leukotoxoid vaccine are excluded from the analysis, there is little change to the risk ratio, with the 95% CI still crossing 1 (I^2=34%; Figure 2). Based on the results of these Forest plots, it would seem that vaccination timing does not impact BRD morbidity in the early feeding period. Looking at total morbidity for each timepoint within the studies, it is nevertheless interesting to note that morbidity was quite high in a number of these studies (Table 3). This is of concern, as it raises the question of whether vaccination at any timepoint is effective. A recent meta-analysis conducted by Theurer comparing vaccination to no vaccination reported a morbidity risk ratio of 0.44, favoring vaccination (95% CI 0.26, 0.74).[20] Still, since the publication of that meta-analysis others have reported no advantage to vaccination, and in fact

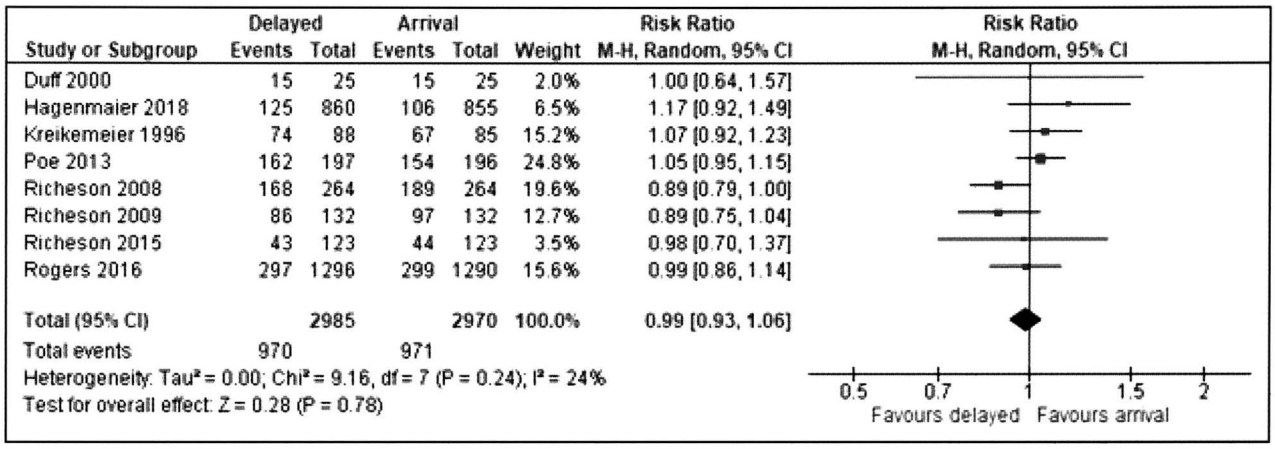

Figure 1. Forest plot of BRD morbidity risk ratios for delayed vs arrival MLV vaccination, all studies.

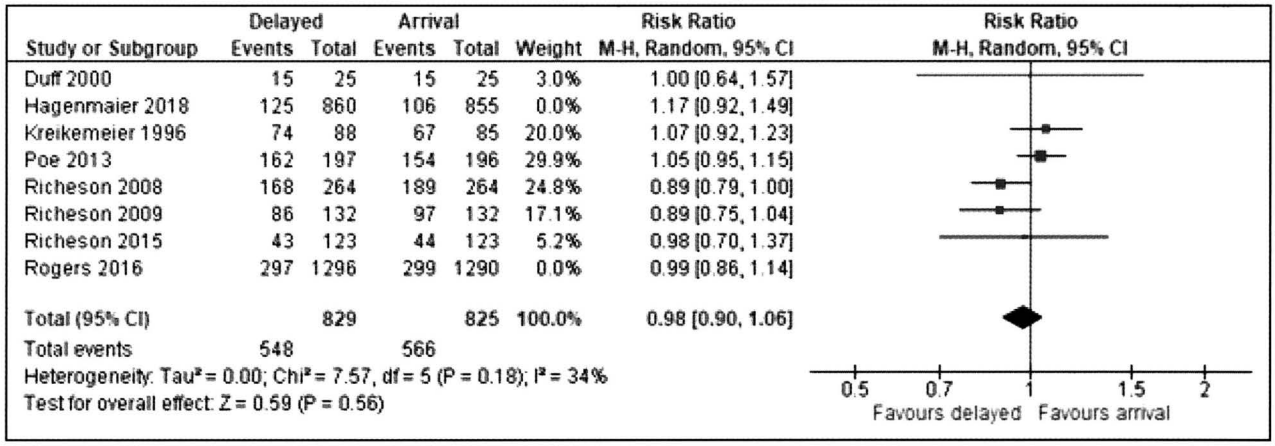

Figure 2. Forest plot of BRD morbidity risk ratios for delayed vs arrival MLV vaccination, excluding studies utilizing *Mh* leukotoxoid.

Table 3. Percent morbidity in evaluated studies for vaccination and arrival.

	Percent morbidity	
Study	**Arrival**	**Delayed**
Duff (2000)	60	60
Hagenmaier (2018)	12	15
Kreikemeier (1996)	79	84
Poe (2013)	79	82
Richeson (2008)	72	64
Richeson (2009)	73	65
Richeson (2015)	36	35
Rogers (2016)	23	23

have reported a disadvantage to vaccination on day 0 versus no vaccination, with a risk ratio of 3.2.[6] The potential increase in morbidity raises concerns regarding current management practices, and warrants further inquiry.

It also seems that retreatment risk does not seem to be impacted by vaccination timing, as the overall risk ratio is 1.01, with a 95% CI 0.91-1.13 (Figure 3). However, it should be mentioned that the model in the Rogers study did show a statistical difference in favor of delayed vaccination in regard to retreatment risk, but meta-analysis does not allow for this, thus, the wider CI seen in our review of the data.[17] When the leukotoxoid vaccine studies are again excluded, the result is similar (Figure 4). For each individual study as well, there is no difference between the timing groups. It would appear that retreatment risk is not impacted by vaccine timing, and as such there may be no difference in disease severity between calves vaccinated at arrival or later.

Regarding mortality, there does appear to be a trend toward delayed vaccination being advantageous; the risk ratio is 0.78 (Figure 5). However, the 95% CI still crossed 1. The overall risk ratio excluding those studies using leukotoxoid is 0.81, with a CI that crosses 1 (Figure 6). Individually, all studies have CI that cross 1 as well, but there is greater variability in the risk ratios for each study. Furthermore, it is possible that had each study reported total deaths from BRD at the end of the feeding period, we may have had more data points. It is not uncommon for unthrifty, chronically ill cattle to linger after treatment before succumbing to death. Had the final BRD mortality been reported, it is possible that

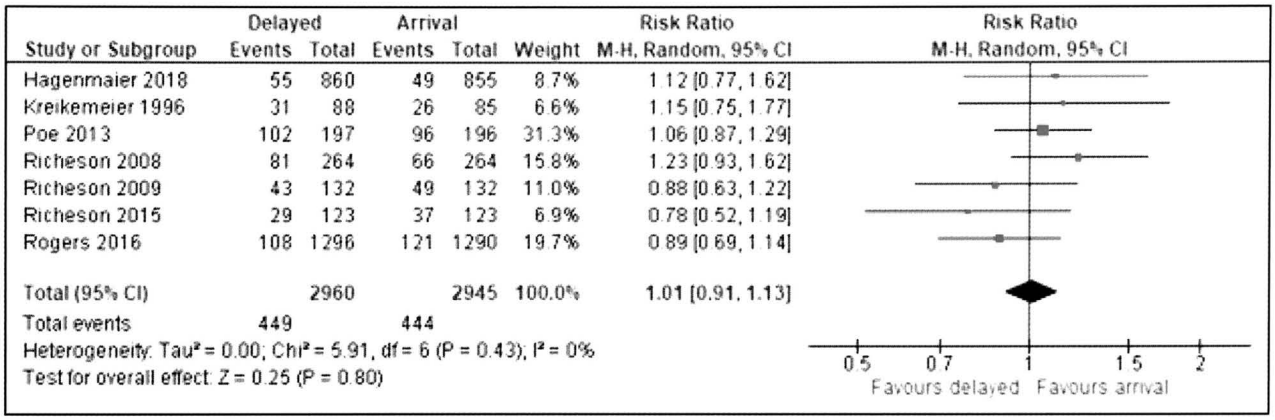

Figure 3. Forest plot of BRD retreatment risk ratios for delayed vs arrival MLV vaccination, all studies.

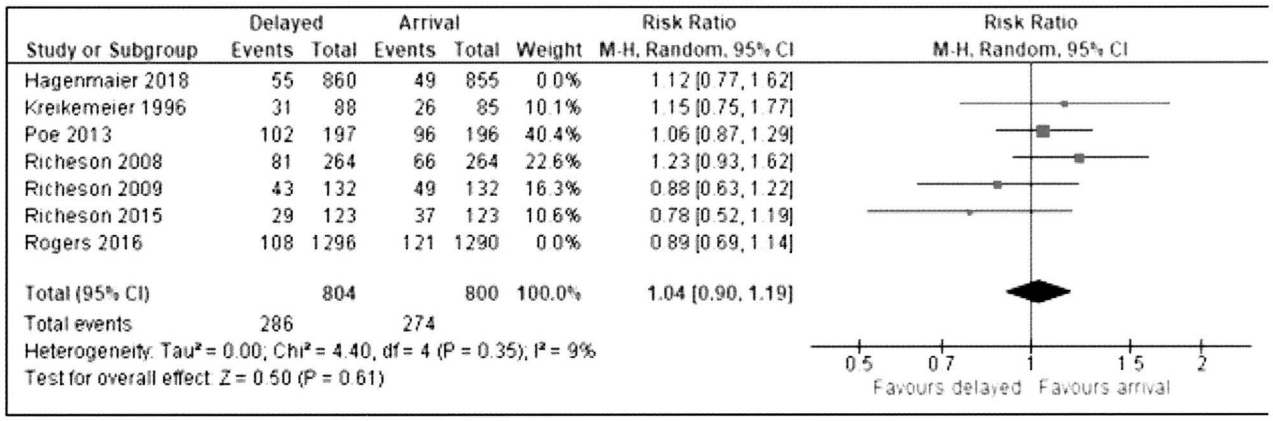

Figure 4. Forest plot of BRD retreatment risk ratios for delayed vs arrival MLV vaccination, excluding studies utilizing *Mh* leukotoxoid.

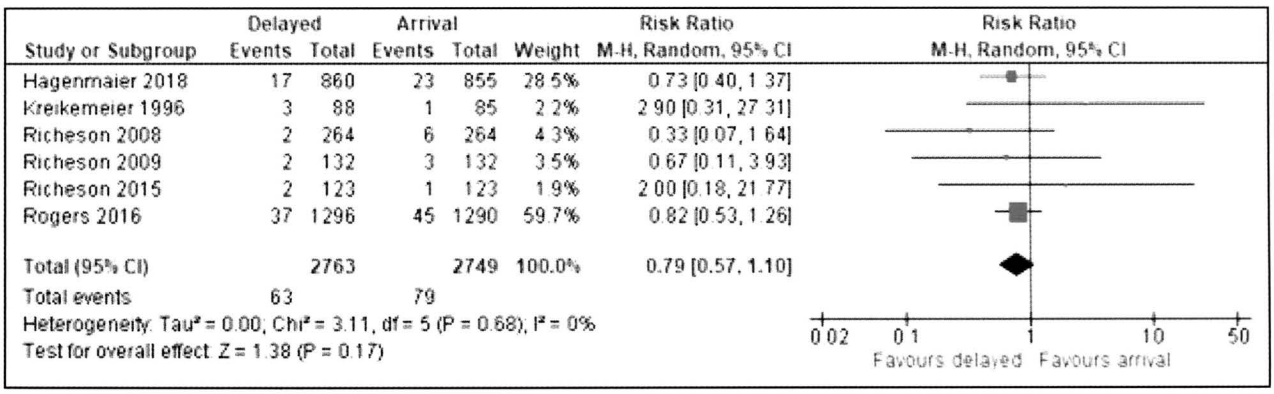

Figure 5. Forest plot of BRD mortality risk ratios for delayed vs arrival MLV vaccination, all studies.

a difference could have been identified between the vaccination timepoints.

Conclusion

Based on the data from the studies analyzed, it would appear that there is not an advantage or disadvantage in terms of morbidity, retreatment risk, or mortality in delaying vaccination. Regardless of vaccine timing, when we consider the high morbidity risk experienced in many of these studies, the use of vaccination as a management tool in this class of cattle demands further evaluation. In light of the results of this meta-analysis, and when we consider the detrimental results of arrival vaccination vs no vaccination as reported by Griffin, it may be that vaccination of high-risk beef cattle in the feedlot or stocker setting is equally ineffective regardless of when it is performed.[6] Indeed, even the study by Duff found no difference in percent morbidity in either arrival or delayed vaccination compared to the unvaccinated control group.[4] More research is needed in larger groups of high-

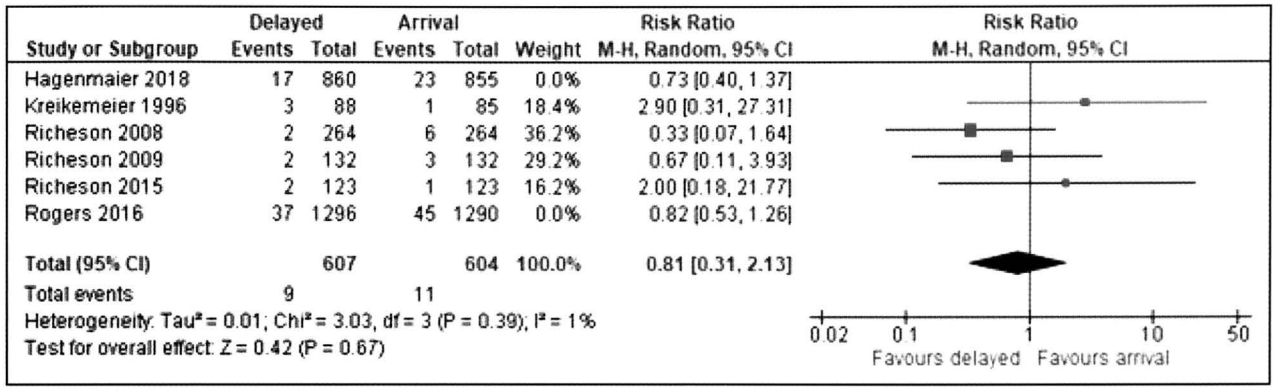

Figure 6. Forest plot of BRD mortality risk ratios for delayed vs arrival MLV vaccination, excluding studies utilizing *Mh* leukotoxoid.

risk cattle with fewer confounding variables to evaluate the timing of vaccination as a factor in the control of bovine respiratory disease.

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Water for Injection	q.s.

pH adjusted with potassium phosphate (monobasic and dibasic).

Gonadorelin is the hypothalamic releasing factor responsible for the release of gonadotropins (e.g., luteinizing hormone [LH], follicle stimulating hormone [FSH]) from the anterior pituitary. Synthetic gonadorelin is physiologically and chemically identical to the endogenous bovine hypothalamic releasing factor.

INDICATIONS FOR USE:

Cystic Ovaries

CYSTORELIN is indicated for the treatment of ovarian follicular cysts in dairy cattle. Ovarian cysts are non-ovulated follicles with incomplete luteinization which result in nymphomania or irregular estrus. Historically, cystic ovaries have responded to an exogenous source of LH such as human chorionic gonadotrophin. CYSTORELIN initiates release of endogenous LH to cause ovulation and luteinization.

Reproductive Synchrony

CYSTORELIN is indicated for use with cloprostenol sodium to synchronize estrous cycles to allow for fixed time artificial insemination (FTAI) in lactating dairy cows and beef cows.

DOSAGE AND ADMINISTRATION:

Cystic Ovaries

The intravenous or intramuscular dosage of CYSTORELIN is 100 mcg gonadorelin diacetate tetrahydrate (2 mL) per cow.

Reproductive Synchrony

The intramuscular dosage of CYSTORELIN is 100 mcg gonadorelin diacetate tetrahydrate (2 mL) per cow, used in reproductive synchrony programs similar to the following:

1. Administer the first CYSTORELIN injection (2 mL) at Time 0.
2. Administer 500 mcg cloprostenol (as cloprostenol sodium) by intramuscular injection 6 to 8 days after the first CYSTORELIN injection.
3. Administer the second CYSTORELIN injection (2 mL) 30 to 72 hours after the cloprostenol sodium injection.
4. Perform FTAI 0 to 24 hours after the second CYSTORELIN injection, or inseminate cows on detected estrus using standard herd practices.

WARNINGS AND PRECAUTIONS:

Not for use in humans.

Keep out of reach of children.

WITHDRAWAL PERIODS:

No withdrawal period or milk discard time is required when used according to the labeling.

The Safety Data Sheet (SDS) contains more detailed occupational safety information. To obtain a SDS or for technical assistance, contact Merial at 1-888-637-4251. To report suspected adverse drug experiences, contact Merial at 1-888-637-4251. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS, or http://www.fda.gov/AnimalVeterinary.

PHARMACOLOGY AND TOXICOLOGY:

Endogenous gonadorelin is synthesized and/or released from the hypothalamus during various stages of the bovine estrus cycle following appropriate neurogenic stimuli. It passes via the hypophyseal portal vessels, to the anterior pituitary to effect the release of gonadotropins (e.g., LH, FSH). Synthetic gonadorelin administered intravenously or intramuscularly also causes the release of endogenous LH or FSH from the anterior pituitary.

Gonadorelin diacetate tetrahydrate has been shown to be safe. The LD50 for mice and rats is greater than 60 mg/kg, and for dogs, greater than 600 mcg/kg, respectively. No adverse effects were noted among rats or dogs administered 120 mcg/kg/day or 72 mcg/kg/day intravenously for 15 days.

It had no adverse effects on heart rate, blood pressure, or EKG to unanesthetized dogs at 60 mcg/kg. In anesthetized dogs it did not produce depression of myocardial or system hemodynamics or adversely affect coronary oxygen supply or myocardial oxygen requirements.

The intravenous administration of 60 mcg/kg/day of gonadorelin diacetate tetrahydrate to pregnant rats and rabbits during organogenesis did not cause embryotoxic or teratogenic effects. Further, CYSTORELIN did not cause irritation at the site of intramuscular administration in dogs with a dose of 72 mcg/kg/day administered for seven (7) days.

TARGET ANIMAL SAFETY:

In addition to the animal safety information presented in the PHARMACOLOGY AND TOXICOLOGY section, the safety of CYSTORELIN was established through the review and evaluation of the extensive published literature available for the use of gonadorelin-containing products.

The intramuscular administration of 1000 mcg gonadorelin diacetate tetrahydrate on five (5) consecutive days to normally cycling dairy cattle had no effect on hematology or clinical chemistries.

In field studies evaluating the effectiveness of CYSTORELIN for the treatment of ovarian follicular cysts, the incidence of health abnormalities was not significantly greater in cows administered CYSTORELIN than cows administered a placebo injection.

The target animal safety of, and injection site reactions to, gonadorelin when used with cloprostenol sodium were evaluated during the conduct of effectiveness field studies. The incidence of health abnormalities was not significantly greater in cows administered gonadorelin than cows administered a placebo injection.

EFFECTIVENESS:

The use of CYSTORELIN for treatment of ovarian follicular cysts in dairy cattle was demonstrated to be effective with a treatment dose of 100 mcg gonadorelin diacetate tetrahydrate.

The effectiveness of gonadorelin for use with cloprostenol sodium to synchronize estrous cycles to allow for FTAI in lactating dairy cows was demonstrated in a field study at 10 different locations in the U.S. Four of the locations represented conditions that would typically cause heat stress in lactating cows. A total of 1607 healthy, non-pregnant, primiparous or multiparous lactating dairy cows within 40-150 days postpartum were enrolled in the study. A total of 805 cows were administered gonadorelin (1 mL; 100 mcg gonadorelin as the acetate salt) and 802 cows were administered an equivalent volume of water for injection as an intramuscular injection twice in the following regimen:

Day 0: 100mcg gonadorelin (as the acetate salt) or sterile water for injection

Day 7: 500 mcg cloprostenol (as cloprostenol sodium)

Day 9: 100mcg gonadorelin (as the acetate salt) or sterile water for injection

Fixed time AI was performed on Day 10, approximately 11 - 31 hours after the Day 9 injection. Cows were evaluated for pregnancy on Day 45 ± 5 days by trans-rectal ultrasound or rectal palpation. Pregnancy rate to FTAI was significantly higher ($P < 0.0001$) in cows treated with gonadorelin (33.4%) than the pregnancy rate to FTAI in cows treated with water (13.6%). The environmental condition (heat stress or not heat stress) did not affect the conclusion of effectiveness. The effectiveness of gonadorelin for use with cloprostenol sodium to synchronize estrous cycles to allow for FTAI in beef cows was demonstrated in a field study at 10 different locations in the U.S. A total of 706 healthy, non-pregnant, primiparous or multiparous beef cows within 40-150 days postpartum were enrolled in the study. A total of 364 cows were administered gonadorelin (1 mL; 100 mcg gonadorelin as the acetate salt) and 342 cows were administered an equivalent volume of water for injection as an intramuscular injection twice in the following regimen:

Day 0: 100mcg gonadorelin (as the acetate salt) or sterile water for injection

Day 7: 500 mcg cloprostenol (as cloprostenol sodium)

Day 9: 100mcg gonadorelin (as the acetate salt) or sterile water for injection

Fixed time AI was performed immediately after the Day 9 injection. Cows were evaluated for pregnancy on Day 55 ± 5 days by trans-rectal ultrasound. Pregnancy rate to FTAI was significantly higher ($P = 0.0006$) in cows treated with gonadorelin (21.7%) than the pregnancy rate to FTAI in cows treated with water (7.4%).

The effectiveness of a 2-mL dose of CYSTORELIN delivering 100 mcg gonadorelin diacetate tetrahydrate (86 mcg gonadorelin) for use with cloprostenol sodium to synchronize estrous cycles to allow for FTAI in lactating dairy cows and beef cows was also demonstrated through references to scientific literature.

HOW SUPPLIED:

CYSTORELIN is available in a concentration of 50 mcg/mL gonadorelin diacetate tetrahydrate (43 mcg/mL gonadorelin) pH adjusted with potassium phosphate (monobasic and dibasic).

CYSTORELIN is supplied in multi-dose vials containing 10 mL and 30 mL of sterile solution.

STORAGE, HANDLING, AND DISPOSAL: Store at or below 77°F (25°C). Brief excursions to 86°F (30°C) are permitted. Use within 6 months of first puncture.

NADA 098-379, Approved by FDA

Marketed by:
Merial, Inc.
Duluth, GA 30096-4640 U.S.A.

SYNCHSURE™

(cloprostenol sodium)

By Merial

Prostaglandin Analogue for Cattle
Equivalent to 250 mcg cloprostenol/mL

CAUTION: Federal law restricts this drug to use by or on the order of a licensed veterinarian.

DESCRIPTION:

SYNCHSURE (cloprostenol sodium) is a synthetic prostaglandin analogue related to prostaglandin $F_{2\alpha}$. SYNCHSURE is indicated for intramuscular use at a two mL dose to induce luteolysis in beef and dairy cattle. The luteolytic action of SYNCHSURE can be used to manipulate the estrous cycle to better fit certain management practices, to terminate pregnancies resulting from mismatings, and to treat certain conditions associated with prolonged luteal function.

USES OF SYNCHSURE:

Unobserved or Nondetected Estrus: If a mature *corpus luteum* is present, SYNCHSURE can be used to induce estrus. Estrus is expected to occur 2 to 5 days following injection. Treated cattle should be inseminated at the usual time following detected estrus or twice at 72 and 96 hours post injection if estrus detection is not possible or desirable.

Pyometra or Chronic Endometritis: Endometritis is inflammation of the uterus and pyometra is characterized by the lack of cyclical estrus behavior and the presence of a persistent *corpus luteum.* SYNCHSURE induces luteolysis which usually results in evacuation of the uterus and a return to normal cycling activity within 14 days after treatment.

Mummified fetus: Induction of luteolysis with SYNCHSURE usually results in the expulsion of the mummified fetus from the uterus. (Manual assistance may be necessary to remove the fetus from the vagina). Normal cyclical activity usually follows.

Luteal Cysts: Luteal cysts may cause abnormal cycling patterns in cows. Treatment with SYNCHSURE can restore normal ovarian activity by causing regression of the luteal cyst.

Pregnancies from mismating: SYNCHSURE can be used to terminate unwanted pregnancies in cattle from 1 week after mating until about 5 months of gestation. The induced abortion is normally uncomplicated and the fetus and placenta are usually expelled 4 to 5 days after the injection. The efficacy of SYNCHSURE in inducing abortion decreases after 5 months of gestation, while the risk of dystocia and additional consequences increases.

Controlled Breeding: SYNCHSURE can be used to schedule estrus and ovulation for individual animals or a group of animals to control breeding times. SYNCHSURE can be used in controlled breeding programs through either single or double injection protocols. Only animals with a mature *corpus luteum* should be treated with the single injection protocol to obtain a maximum response to the single injection. Prior to treatment, cattle should be examined rectally and found to be anatomically normal and nonpregnant. Before a controlled breeding program is planned, the producer and his consulting veterinarian should review the operation's breeding history, herd health and nutritional status and agree that a controlled breeding program is practical in that particular situation.

The use information provided here is not comprehensive. Talk to your veterinarian and consult the full prescribing information available at www.synchsure.com for further details on uses of SYNCHSURE.

SAFETY AND TOXICITY: AT 50 and 100 times the recommended dose, mild side effects may be detected in some cattle including increased uneasiness, slight frothing, and milk let-down. The risk information provided here is not comprehensive. To learn more, talk to your veterinarian about SYNCHSURE or call 1-888-637-4251. The full prescribing information can be found at www.synchsure.com.

CONTRAINDICATIONS: SYNCHSURE should not be given to pregnant animals whose calf is not meant to be aborted.

WARNINGS: For animal use only. Do not use in humans. Keep out of reach of Children. Women of childbearing age, asthmatics and persons with respiratory problems should exercise extreme caution with handling this product. In early stages, women may not be aware of their pregnancies. SYNCHSURE is readily absorbed through the skin and may cause abortion and/or bronchiospasms: direct contact with the skin should be avoided. Accidental spillage on the skin should be washed off immediately with soap and water.

PRECAUTIONS:

Careful aseptic techniques should be employed to decrease the possibility of post-injection bacterial infection. Antibiotic therapy should be employed at the first sign of infection. The Safety Data Sheet (SDS) contains more detailed occupational safety information. For technical assistance, to request an SDS, or to report a suspected adverse event, contact Merial Technical Support at 1-888-637-4251. For additional information about adverse event reporting for animal drugs, contact FDA at 1-888-FDA-VETS, or http://www.fda.gov/AnimalVeterinary.

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MERIAL

1050-2907-0A
Rev. 12/2017

CONGRATS, IT'S A CALF! AGAIN!

It's easy to be confident that your cows will get pregnant when you use Cystorelin® (gonadorelin) and Synchsure™ (cloprostenol sodium) together. They're an effective combination for reproductive efficiency. So, after use, this test is more of a formality.

MAXIMIZE REPRODUCTIVE EFFICIENCY ON YOUR OPERATION AT SYNCTHEHERD.COM.

IMPORTANT SAFETY INFORMATION FOR CYSTORELIN: Do not use in humans. Keep this and all drugs out of the reach of children.

IMPORTANT SAFETY INFORMATION FOR SYNCHSURE: FOR ANIMAL USE ONLY, NOT FOR HUMAN USE. KEEP OUT OF REACH OF CHILDREN. Women of child-bearing age, asthmatics, and persons with bronchial and other respiratory problems should exercise extreme caution when handling this product. In the early stages women may be unaware of their pregnancies. SYNCHSURE is readily absorbed through the skin and may cause abortion and/or bronchospasms: direct contact with the skin should therefore be avoided. Accidental spillage on the skin should be washed off immediately with soap and water.

Boehringer Ingelheim

Cattle First.

CYSTORELIN® (gonadorelin) By Merial

SYNCHSURE™ (cloprostenol sodium) By Merial