Effects of administration of a modified-live virus respiratory vaccine and timing of vaccination on health and performance of high-risk beef stocker calves

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Abstract

A randomized, controlled field study was conducted to evaluate the efficiency of various management regimens using a pentavalent modified-live virus (MLV) respiratory vaccine containing bovine herpesvirus-1, bovine viral diarrhea virus types 1 and 2, parainfluenza-3 virus, and bovine respiratory syncytial virus. Crossbred beef calves (n = 370) weighing 466 ± 5.5 lb (212 ± 2.5 kg) were acquired from auction markets. Calves were stratified by castrate status on arrival and assigned randomly to 1 of 3 experimental treatments: 1) initial MLV vaccine on day 0 (AMLV), 2) initial MLV vaccine on day 14 (DMLV), or 3) no vaccination with MLV vaccine until the end of the 42-day receiving period (NMLV). Least-squares means were evaluated using the contrasts: 1) vaccinated (VAC) vs NMLV, and 2) AMLV vs DMLV. Body weight was recorded on days 0, 14, 28, 42, and at the end of the grazing period, and health was monitored daily. Average daily gain was greater for DMLV vs AMLV from days 14 to 28 (P<0.01), and VAC vs NMLV from days 28 to 42 (P = 0.04). However, performance was not different (P ≥ 0.16) between VAC and NMLV from days 0 to 42. The overall incidence of clinical bovine respiratory disease did not differ (P ≥ 0.82); however, there was a tendency (P = 0.08) for increased relapse rate in NMLV calves compared to VAC. Results suggest that MLV vaccine administered to high-risk stocker calves on day 0 or 14 may reduce relapse rate; whereas, gain was reduced transiently when MLV vaccine was administered at day 0.

Key words: bovine respiratory disease, BRO, health, performance, vaccination

Résumé

Un essai contrôlé avec répartition aléatoire sur le terrain a été mené pour évaluer l'efficacité de différents protocoles de gestion avec un vaccin respiratoire pentavalent avec virus vivants modifiés comportant l'herpès-virus bovin, le virus de la diarrhée virale bovine de type 1 et 2, le virus parainfluenza 3 bovin et le virus respiratoire syncytial bovin. Des veaux de boucherie de race croisée (n=370) pesant 466 ± 5.5 lb (212 ± 2.5 kg) ont été obtenus à l'encan. Les veaux ont été stratifiés à leur arrivée selon leur statut de castration et ont été assignés aléatoirement dans trois traitements expérimentaux : 1) administration initiale du vaccin pentavalent au jour 0 (AMLV), 2) administration initiale du vaccin pentavalent au jour 14 (DMLV), ou 3) sans vaccination jusqu'au jour 42 de la période de réception (NMLV). Les moyennes de moindres carrés ont été évaluées avec des contrastes : 1) vacciné (VAC) vs NMLV et 2) AMLV vs DMLV. Le poids corporel a été pris aux jours 0, 14, 28, 42 et à la fin de la période en pâturage tandis que la santé était évaluée à tous les jours. Le gain moyen quotidien était plus élevé dans le traitement DMLV que dans le traitement AMLV du jour 14 au jour 28 (P<0.01) et plus élevé dans le traitement VAC que dans le traitement NMLV du jour 28 au jour 42 (P=0.04). Toutefois, il n'y avait pas de différence au niveau de la performance (P≥0.16) entre les traitements VAC et NMLV du jour 0 au jour 42. L'incidence globale du complexe respiratoire bovin n'était pas différente (P≥0.82). Néanmoins, le taux de rechute était marginalement (P=0.08) plus élevé dans le traitement à NMLV que dans le traitement VAC. Les résultats suggèrent que l'administration d'un vaccin pentavalent avec virus vivants modifiés chez les veaux d'élevage à haut risque à leur arrivé pourrait réduire le taux de rechute. Le gain était moindre chez les veaux recevant le vaccin au jour 0.

Introduction

Bovine respiratory disease (BRD) is a complicated syndrome that may result from numerous predisposing factors which cause immune dysfunction, acute infection with 1 or
more viral agents, and ultimately lower respiratory infection with bacteria that commonly inhabit the nasopharynx of healthy animals. It is routine practice for stocker and feedlot operators to administer a parenteral multivalent modified-live virus (MLV) respiratory vaccine during initial processing with the goal of stimulating a systemic immune response against viral agents involved in BRD. Evidence to support the validity of this practice in high-risk, newly received calves is limited. A vaccine is considered efficacious provided it is shown to be biologically active and safely stimulates an active immune response against the agents contained in the vaccine. For a vaccine to demonstrate field efficiency, it should result in a significant reduction in clinical illness, improvement in weight gain, and a clear economic advantage (cost:benefit) in the commercial production setting. Although vaccination with a multivalent MLV vaccine has repeatedly shown efficacy in controlled challenge studies, the efficiency of MLV vaccination during natural disease epidemics is less apparent. Previous literature reviews suggest that evidence for vaccine efficacy in newly arrived cattle at a feedlot facility is limited. Recent studies have evaluated the timing of vaccination, effects of revaccination, or compared different vaccine products; however, a negative control treatment is rarely used in field studies. Our objective was to evaluate the effect of parenteral MLV vaccine administration and timing on health and performance of high-risk, newly received beef stocker calves.

Materials and Methods

Treatment Assignment and Processing

Animal methods were approved by the University of Arkansas Animal Care and Use Committee (Protocol # 06066). Vaccines and antimicrobials used in this study were administered according to Beef Quality Assurance guidelines. Crossbred bull (n = 169) and steer (n = 201) beef calves (Fall, n = 184, initial BW = 464 ± 5.7 kg (211 ± 2.6 kg); Spring, n = 186, initial BW = 469 ± 11.9 kg (213 ± 5.4 kg)) were purchased from a northern Arkansas auction barn and shipped approximately 37 miles (60 km) to the University of Arkansas Livestock and Forestry Research Station (LFRS) near Batesville, AR. Cattle were received on 2 separate dates in the fall (September 12, 2009 (Block 1, n = 93) and September 19, 2009 (Block 2, n = 91)), and 2 separate dates in the spring (January 14, 2010 (Block 3, n = 71) and January 19, 2010 (Block 4, n = 118)).

Calves were assigned to vaccine treatment according to on-arrival castrate status (bull or steer) because it has been reported that bulls are at greater risk for development of clinical signs of BRD as compared to steer cohorts. Castrate status was evenly distributed by assigning a similar number of bull and steer calves to each vaccine treatment. Following initial processing on day 0, calves were placed in pens by treatment with 4 pens per treatment for each of 2 seasons. This resulted in a total of 8 pen replicates/treatment. The 3 vaccine treatments included: 1) initial vaccination with MLV vaccine on day 0 (AMLV), 2) initial vaccination with MLV vaccine on day 14 (DMLV), or 3) no vaccination with MLV vaccine until the end of the 42-day receiving period (NMLV). The AMLV calves were administered a pentavalent vaccinea containing live-attenuated strains of bovine herpesvirus-1, bovine viral diarrhea virus types 1 and 2, parainfluenza-3 virus, and bovine respiratory syncytial virus in combination on day 0. Cattle assigned to the DMLV treatment did not receive their initial MLV vaccination until day 14 of study, and NMLV did not receive MLV vaccination until the end of the 42-day receiving period. Also on day 0, calves were weighed, administered a multivalent clostridial bacterin with tetanus toxoidb, treated for internal and external parasites, and bull calves were castrated using a rubber banding device. Multiple-dose vaccination against viruses known to contribute to BRD is common; therefore, AMLV and DMLV were administered 2 injections of the MLV respiratory vaccine 14 days apart according to the previously described schedule. In addition, cattle were given metaphylaxis with tilmicosin phosphate according to procedures previously described. A 24-hour post-metaphylaxis interval was implemented; calves were observed for signs of BRD and eligible for treatment beginning on day 1. After cattle were sorted, they were moved to 1.0 acre (0.4 hectare) pens and provided a 22% crude protein (CP) supplement based on corn gluten feed at 1% of bodyweight (dry matter (DM) basis) and free-choice access to bermudagrass hay (10% CP, 56% total digestible nutrients) for the entire 42-day receiving period.

To determine differences in gain performance, cattle were weighed (un-shrunken) at 14-day intervals during the receiving period (days 14, 28, and 42). Cattle were shrunken and weighed at the beginning (day 43) and end of the grazing period. On day 14, all cattle were revaccinated with the clostridial bacterin with tetanus toxoid, AMLV cattle were revaccinated with the pentavalent MLV vaccine, and DMLV cattle received initial vaccination with pentavalent MLV vaccine. Two weeks later (day 28), DMLV were revaccinated with the pentavalent MLV vaccine.

Evaluation of BRD

Calves were observed each morning (0800) by LFRS personnel for signs consistent with respiratory illness (depression, lethargy, rapid breathing, nasal or ocular discharge, and lack of appetite) and other conditions which may have had animal welfare implications. Morbidity investigators were blinded to treatment pen allotment. Cattle observed with ≥ 2 visual signs of BRD were removed from their pen, restrained, and rectal temperature (RT) was determined using a digital thermometer. Calves with RT ≥ 104°F (40°C) were considered morbid and were treated with an antimicrobial according to a pre-determined protocol specific for the current study. The treatment protocol included initial antimicrobial therapy with florfenicol at 6 mL/100 lb (45.4 kg) BW. Cattle were eligible for re-evaluation after expiration of a 72-hour post-treatment interval (PTI), and those with ≥ 2 visual signs of BRD morbidity and a rectal temperature of...
 ≥ 104°F (40°C) were considered morbid a second time and administered a second antibiotic treatment using enrofloxacinb at 5 mL/100 lb (45.4 kg) BW. Subsequent pulls meeting the BRD case definition after a 72-hour PTI were administered 1.5 mL/100 lb (45.4 kg) BW of tilmicosin phosphatec for the third treatment, ceftiofur crystalline-free acidd at 1.5 mL/100 lb (45.4 kg) BW for the fourth treatment, and tulathromycine at 1.1 mL/100 lb (45.4 kg) BW for the fifth and final treatment. Cattle were returned to their respective home pen immediately following each antimicrobial treatment. Treatment data recorded for individual animals included treatment date and amount, type, and cost of antibiotic administered.

Grazing Period

Post-receiving performance was evaluated based on shrunk body weight recorded following a 16-hour fast on day 43 and shrunk body weight following termination of grazing of cool-season annual or perennial pastures when forage availability became limited. Steers were implanted with 40 mg trenbolone acetate and 8 mg estradiolk at the initiation of grazing. Pastures were wheat (Triticum aestivum L, cv Roane, 295 lb or 134 kg/ha) planted into dedicated crop fields, toxic endophyte-infected (Neotyphodium coenophialum, N. lolii) or non-toxic endophyte-infected tall fescue (Festuca arundinacea Shreb.) pastures. Stocking rates were 1.5 steers/acre (3.7 steers/hectare) in the fall and 2 steers/acre (4.9 steers/hectare) in the spring. Steers from each vaccination treatment were similarly represented in pastures of each forage type. In both the fall and spring, pastures were stocked when forage height reached approximately 8 inches (20 cm). The grazing method was continuous stocking, with steers having access to all of each assigned pasture continuously for a 98-day grazing period for the fall (Blocks 1 and 2) and a 62-day grazing period for the spring (Blocks 3 and 4). Cattle were removed (end of grazing period) when forage mass reached <890 lb DM/acre (<1,000 kg DM/hectare).

Statistical Analysis

Experimental treatments were arranged in a randomized complete block design. Pen was identified as the experimental unit. Date of shipment arrival (block) was considered a random effect in the model. Pen within block was used as the denominator mean square for the treatment effect test. Gain performance data, days to first pull, and treatment cost were analyzed using the PROC MIXED procedure of SAS1; whereas, morbidity and relapse rate was analyzed using PROC GLIMMIX. Castrate status upon arrival and BRD vaccination treatment were included as fixed effects in the model and block was considered a random effect. Least-squares means of dependent variables were separated using preplanned contrasts consisting of vaccinated (VAC; AMLV and DMLV) vs NMLV, and AMLV vs DMLV. For results of the contrast comparisons, a P-value ≤ 0.05 was considered statistically significant; whereas, a tendency was considered for a contrast P-value > 0.05 and ≤ 0.10.

Results and Discussion

Growth Performance

Performance data are presented in Table 1. No differences in body weight were recorded during the 42-day receiving period (P ≥ 0.46). However, ADG was greater for DMLV compared to AMLV from days 14 to 28 (1.43 and 1.03 lb/day; 0.65 and 0.47 kg/day, respectively; P < 0.01). A difference (P = 0.03) in ADG for this contrast comparison was also observed during the subsequent grazing period; DMLV and AMLV gained 2.38 and 2.18 lb/day (1.08 and 0.99 kg/day), respectively.

### Table 1. Effect of modified-live virus respiratory vaccine regimen on growth performance of high-risk stocker calves.

<table>
<thead>
<tr>
<th>Item</th>
<th>NMLV (n=8)</th>
<th>AMLV (n=8)</th>
<th>DMLV (n=8)</th>
<th>SE†</th>
<th>NMLV vs VAC</th>
<th>AMLV vs DMLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, lb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>469</td>
<td>466</td>
<td>469</td>
<td>5.7</td>
<td>0.90</td>
<td>0.71</td>
</tr>
<tr>
<td>Day 14</td>
<td>497</td>
<td>495</td>
<td>495</td>
<td>7.0</td>
<td>0.76</td>
<td>0.98</td>
</tr>
<tr>
<td>Day 28</td>
<td>513</td>
<td>510</td>
<td>515</td>
<td>11.9</td>
<td>0.97</td>
<td>0.49</td>
</tr>
<tr>
<td>Day 42</td>
<td>526</td>
<td>526</td>
<td>530</td>
<td>12.5</td>
<td>0.62</td>
<td>0.46</td>
</tr>
<tr>
<td>Average daily gain, lb/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0 to 14</td>
<td>1.98</td>
<td>2.05</td>
<td>1.85</td>
<td>0.48</td>
<td>0.83</td>
<td>0.28</td>
</tr>
<tr>
<td>Day 14 to 28</td>
<td>1.12</td>
<td>1.03</td>
<td>1.43</td>
<td>0.51</td>
<td>0.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Day 28 to 42</td>
<td>0.90</td>
<td>1.14</td>
<td>1.17</td>
<td>0.13</td>
<td>0.04</td>
<td>0.86</td>
</tr>
<tr>
<td>Day 0 to 42</td>
<td>1.34</td>
<td>1.41</td>
<td>1.47</td>
<td>0.33</td>
<td>0.16</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*Treatments were no vaccination with a respiratory vaccine during the 42-day receiving period (NMLV), or initial vaccination with a pentavalent (bovine herpesvirus-1, bovine viral diarrhea virus type 1 and 2, parainfluenza-3 virus, and bovine respiratory syncytial virus) modified-live virus respiratory vaccine (Bovi-shield Gold® 5; Zoetis) at initial processing (day 0; AMLV) or on day 14 (DMLV). The AMLV and DMLV treatment groups were revaccinated 14 days following initial vaccination.

†Standard error of the least-squares means.

‡Pre-planned contrast coefficients used to separate least-squares means included: NMLV vs vaccinated (VAC; AMLV and DMLV), and AMLV vs DMLV.
Previous studies evaluating gain performance in high-risk stocker or feedlot cattle administered on-arrival or delayed vaccination regimens are limited. Similar to ADG results in the current study, Richeson et al.8 observed an improvement in performance from day 0 to 14 and during the entire 42-day receiving period, but not during the subsequent grazing period for high-risk stocker calves administered the delayed procedure (initial MLV on day 14). Several factors associated with on-arrival vaccination could decrease performance in high-risk cattle. The animals are likely experiencing the greatest physiological stress during initial processing (day 0) and for at least several days thereafter. Additional physiological stress at this time contributed by on-arrival administration of a MLV respiratory vaccine could additively impact endocrine and/or acute-phase responses1 which are known to be both catabolic and metabolically demanding. Furthermore, introducing live-attenuated virus strains in animals more likely to be acutely infected with wild-type virus(es) transmitted during the marketing process could result in immune taxation, complicating the immune system of the animal during this critical time.11 However, one could certainly question the biological significance or repeatability of depressed performance attributed to on-arrival MLV vaccination in high-risk calves; the ADG between NMLV and AMLV from day 14 to 28 was numerically greater for NMLV, but the difference was less pronounced compared to the contrast between AMLV and DMLV during this interim period. Also, when comparing NMLV to VAC, the NMLV treatment gained less ($P = 0.04$) from day 28 to 42. Perhaps most clear from the current study and previous reports in the literature is the lack of gain performance benefit in high-risk stocker cattle receiving a MLV respiratory vaccine on arrival, which is an important component of vaccine efficiency. Further research is needed to improve understanding of the potential for metabolic cost of MLV respiratory vaccination in animals experiencing physiological stress.

### Health

There were no significant differences for any of the health parameters evaluated in this study ($P \geq 0.08$; Table 2). However, there was a tendency ($P = 0.10$) for more NMLV cattle requiring 2 antimicrobial treatments and relapse rate tended to be greater ($P = 0.08$) for NMLV (44.2%) compared to VAC (26.7%). Likewise, there was a numerical, but not a statistical increase ($P = 0.11$) in the percentage of NMLV treated 3 times with an antimicrobial. The percentage of cattle treated 3 times was 5.2, 0.5, and 3.6% for NMLV, AMLV and DMLV vaccine groups, respectively (data not shown). Furthermore, overall BRD morbidity was not different ($P = 0.82$), which averaged 34.0% for NMLV, 35.4% for AMLV, and 34.9% for DMLV (Table 2; Figure 1). The overall morbidity results are in general agreement with similar studies evaluating on-arrival vs delayed MLV respiratory vaccination in high risk calves. Richeson et al.8 reported morbidity was similar in calves administered their initial MLV respiratory vaccine on day 0 (arrival) or 14 (71.5 vs 63.5%). Another study9 evaluated the delayed administration of MLV respiratory vaccine, clostridial bacterin, or both, and no differences in BRD.

### Table 2. Effect of modified-live virus respiratory vaccine regimen on health and treatment cost of high-risk stocker calves.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment*</th>
<th>Contrast, $P$‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NMLV (n=8)</td>
<td>AMLV (n=8)</td>
</tr>
<tr>
<td>BRD morbidity§, %</td>
<td>34.0</td>
<td>35.4</td>
</tr>
<tr>
<td>Relapse¶, %</td>
<td>44.2</td>
<td>30.1</td>
</tr>
<tr>
<td>Days to 1st treatment</td>
<td>10.8</td>
<td>9.9</td>
</tr>
<tr>
<td>Mortality, %</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Treatment cost¶, $/steer</td>
<td>15.50</td>
<td>14.12</td>
</tr>
</tbody>
</table>

* Treatments were no vaccination with a respiratory vaccine during the 42-day receiving period (NMLV), or initial vaccination with a pentavalent (bovine herpesvirus-1, bovine viral diarrhea virus types 1 and 2, parainfluenza, virus, and bovine respiratory syncytial virus) modified-live virus respiratory vaccine (Bovi-Shield Gold® 5; Zoetis) at initial processing (day 0; AMLV) or on day 14 (DMLV). The AMLV and DMLV treatment groups were revaccinated 14 days following initial vaccination.

† Standard error of the least-squares means.

‡ Pre-planned contrast coefficients used to separate least-squares means included: NMLV vs vaccinated (VAC; AMLV and DMLV), and AMLV vs DMLV.

§ Percentage of total population diagnosed and treated at least 1 time. Cattle observed with 22 signs consistent with respiratory disease and rectal temperature ≥104°F (40°C) were diagnosed with clinical BRD and medicated with florfenicol (Nuflor®, Merck Animal Health) at 6.0 ml/100 lb (45.4 kg) BW (first treatment); enrofloxacin (Baytril®, Bayer Animal Health) at 4.0 ml/100 lb (45.4 kg) BW (second treatment); tilmicosin (Micotil®, Elanco Animal Health) at 1.5 ml/100 lb (45.4 kg) BW (third treatment); ceftiofur (Excede®, Zoetis) at 1.5 ml/100 lb (45.4 kg) BW (fourth treatment); and tulathromycin (Draxxin®, Zoetis) at 1.1 ml/100 lb (45.4 kg) BW (fifth treatment).

¶ The percentage of cattle that were diagnosed and treated for BRD requiring an additional treatment or BRD. An animal that relapsed more than 1 time was only counted once.

† Treatment cost for BRD (no chute fee was assigned) assuming the following fixed antimicrobial costs: tilmicosin at $1.07/ml, florfenicol at $0.50/ml, enrofloxacin at $0.68/ml, ceftiofur at $1.67/ml, and tulathromycin at $3.00/ml.
morbidity were observed. Likewise, Duff et al.\textsuperscript{4} evaluated the effects of vaccine administration (intranasal vs intramuscular vs unvaccinated control) in newly received beef calves, and did not observe differences in morbidity. Trends observed in the current study for increased secondary disease variables for NMLV suggest that: 1) MLV respiratory vaccination of newly received beef calves was effective under current study conditions, and 2) challenges exist with sample size when comparing pen means of proportional data, and risk of type II error exists.

The slight increase in the number of NMLV calves treated with an antimicrobial resulted in a correspondingly slight increase in antimicrobial treatment cost ($/steer). The antimicrobial treatment cost was $1.36/steer more for NMLV when contrasted against the mean treatment cost of VAC; however, this difference was not significant ($P = 0.26$). Nevertheless, it is worth noting that the relatively inexpensive cost of MLV respiratory vaccine (typically ≤$1.00/dose) was similar to the numerical increase in antimicrobial treatment cost for NMLV ($1.36/steer). Also, the MLV respiratory vaccine used in the current study did not contain bacterin or toxoid agents intended to protect against bacteria or leukotoxin known to be involved in BRD pathogenesis. Combination vaccine/bacterins may or may not affect clinical BRD morbidity in the field, yet inclusion of a bacterin significantly increases vaccine cost.

**Conclusions**

The timing of MLV respiratory vaccination affected gain performance and health of high-risk stocker calves used in this study. There was an increase in pasture ADG and a transient improvement in gain during the 42-day receiving period realized for the delayed procedure, yet no differences in gain were observed over the entire 42-day receiving period. Morbidity or mortality associated with clinical BRD was not clearly affected by MLV vaccination timing. Furthermore, BRD morbidity during the receiving period was not affected by administration of a MLV respiratory vaccine; however, there was a trend towards a higher relapse rate in the control group while antimicrobial treatment cost did not differ statistically. Calves acquired from auction barns are likely to experience stress-induced immune dysfunction and natural exposure to viral agent(s) before initial processing at a stocker or feedlot facility. Practitioners should continue to encourage their cow-calf clients to vaccinate calves before marketing with the goal of developing protective immunity prior to experiencing chronic stress and wild-type virus exposure. Other preconditioning management factors implemented at the ranch origin, including castration, weaning, and bunk training, are likely to affect subsequent health and performance outcomes in newly received beef calves.

**Endnotes**

\textsuperscript{a}Bovi-Shield Gold\textsuperscript{®} 5, Zoetis, Kalamazoo, MI  
\textsuperscript{b}Covexin-8\textsuperscript{®}, Merck Animal Health, Summit, NJ  
\textsuperscript{c}Cydectin\textsuperscript{®}, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO  
\textsuperscript{d}The California Bander, InoSol, Co. LLC, El Centro, CA  
\textsuperscript{e}Micotil\textsuperscript{®}, Elanco Animal Health, Indianapolis, IN  
\textsuperscript{f}Model No. M216, GLA Agricultural Electronics, San Luis Obispo, CA  
\textsuperscript{g}Nuflor\textsuperscript{®}, Merck Animal Health, Summit, NJ  
\textsuperscript{h}Baytril\textsuperscript{®}, Bayer Animal Health, Shawnee Mission, KS  
\textsuperscript{i}Excede\textsuperscript{®}, Zoetis, Kalamazoo, MI  
\textsuperscript{j}Draxxin\textsuperscript{®}, Zoetis, Kalamazoo, MI  
\textsuperscript{k}Revalor\textsuperscript{®}-G, Merck Animal Health, Summit, NJ  
\textsuperscript{l}SAS version 9.3, SAS Inst., Inc., Cary, NC

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