Date of Approval: April 2, 2020

CORRECTED FREEDOM OF INFORMATION SUMMARY

APPLICATION FOR CONDITIONAL APPROVAL

Application Number 141-527

Baytril® 100-CA1

enrofloxacin

Injectable Solution

Cattle: replacement dairy heifers under 20 months of age and all classes of beef cattle except beef calves less than 2 months of age and beef bulls intended for breeding (any age); not for use in any other class of dairy cattle or in veal calves

For the treatment of clinical anaplasmosis associated with *Anaplasma marginale* in replacement dairy heifers under 20 months of age and all classes of beef cattle except beef calves less than 2 months of age and beef bulls intended for breeding (any age). Not for use in any other class of dairy cattle or in veal calves.

Sponsored by:

Elanco US, Inc.

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I. GENERAL INFORMATION

Baytril® 100-CA1 is conditionally approved for the treatment of clinical anaplasmosis associated with *Anaplasma marginale* in replacement dairy heifers under 20 months of age and all classes of beef cattle except beef calves less than 2 months of age and beef bulls intended for breeding (any age); not for use in any other class of dairy cattle or in veal calves. This qualifies as a minor use in a major species because the number of cattle that would be treated for clinical anaplasmosis is estimated to be fewer than 310,000 cattle in the U.S. each year[1].

A. File Number

Application Number 141-527

B. Sponsor

Elanco US, Inc.
2500 Innovation Way
Greenfield, IN 46140

Drug Labeler Code: 058198

C. Proprietary Name

Baytril® 100-CA1

D. Drug Product Established Name

Enrofloxacin

E. Pharmacological Category

Antimicrobial

F. Dosage Form

Injectable solution

G. Amount of Active Ingredient

100 mg/mL

H. How Supplied

250 mL bottle

I. Dispensing Status

Rx

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[1] See additional information in Section VI. Agency Conclusions

J. Dosage Regimen

Baytril® 100-CA1 should be administered as a single dose for treatment of clinical anaplasmosis. Administer, by subcutaneous injection, a single dose of 12.5 mg/kg of body weight (5.7 mL/100 lb). Administered dose volume should not exceed 20 mL per injection site.

K. Route of Administration

Subcutaneous

L. Species/Class

Cattle: replacement dairy heifers under 20 months of age and all classes of beef cattle except beef calves less than 2 months of age and beef bulls intended for breeding (any age); not for use in any other class of dairy cattle or in veal calves

M. Indication

For the treatment of clinical anaplasmosis associated with *Anaplasma marginale* in replacement dairy heifers under 20 months of age and all classes of beef cattle except beef calves less than 2 months of age and beef bulls intended for breeding (any age). Not for use in any other class of dairy cattle or in veal calves.

II. EFFECTIVENESS

Conditional Dose: The conditional dose for the indication "for the treatment of clinical anaplasmosis in cattle" is a single subcutaneous (SC) dose of 12.5 mg enrofloxacin/kg body weight (BW). The safety data and the data to demonstrate reasonable expectation of effectiveness provide support for this conditional dose.

A. Dosage Characterization

This conditional approval provides for a single SC dose of 12.5 mg enrofloxacin/kg BW, which is the same as the previously-approved single-dose enrofloxacin regimen for Baytril® 100, approved under NADA 141-068 for the treatment of bovine respiratory disease in cattle. The Freedom of Information (FOI) Summary for the original approval of NADA 141-068 dated July 24, 1998, contains dosage characterization information for cattle.

B. Reasonable Expectation of Effectiveness

A combination of published literature and reports from studies conducted by the sponsor was used to demonstrate reasonable expectation of effectiveness for the use of enrofloxacin for the treatment of clinical anaplasmosis in cattle when administered as a single SC dose of 12.5 mg enrofloxacin/kg BW. The three most relevant sources of information are summarized below.

1. Evaluation of the Therapeutic Efficacy of Products Containing Active Enrofloxacin and Oxytetracycline versus *Anaplasma marginale* Living as Experimentally Infected Bovine Parasites. Sponsor Report ID 041722. March 2015.

A study was conducted in Brazil to evaluate the effectiveness of enrofloxacin compared to oxytetracycline in experimentally-induced anaplasmosis. Sixteen male and female cattle (9 months old, approximately 150-250 kg BW), were used in the study. On Day 0, animals were inoculated with erythrocytes containing a single strain of *A. marginale* obtained from a natural infection, to induce parasitemia. Following inoculation, animals were observed once daily to detect the onset of clinical anaplasmosis. Rectal temperature, hematocrit (packed cell volume, PCV), and parasitemia (percent parasitized erythrocytes, PPE) evaluations were made at scheduled intervals during the study. When clinical anaplasmosis was detected (rectal temperature ≥ 39.5 °C, PPE ≥ 2%, and PCV ≥ 20% reduction from baseline), animals were treated with enrofloxacin (four animals, 3 mL/40 kg BW intramuscularly once [7.5 mg/kg BW]), oxytetracycline (two regimens, four animals each), or left as untreated controls (four animals). In the enrofloxacin-treated group, PCVs were increased, rectal temperatures returned to normal, and parasitemia was decreased in all four animals within 96 to 120 hours after treatment. The mean PPE in the enrofloxacin-treated group was numerically lower than the control group from 24 hours to 120 hours after treatments. The study demonstrates that administration of enrofloxacin was successful in reducing parasitemia and improving clinical signs in cattle with clinical anaplasmosis. Because this lower single dose was effective, a higher single dose (i.e., the proposed dose of 12.5 mg/kg BW) is also reasonably expected to be effective. Although a different administration route (IM) was used in this study, another evaluated literature reference (as described below in III.B.2.) showed beneficial results using the intended (SC) route of administration.

2. Coetzee, J.F., and Apley, M.D., 2006. Efficacy of Enrofloxacin against Severe Experimental *Anaplasma marginale* Infections in Splenectomized Calves. *Veterinary Therapeutics* 7(3), 319-328.

   This study evaluated the effectiveness of enrofloxacin in treating *A. marginale* infections in severely infected animals. Four splenectomized Holstein calves (5 to 8 months old) were inoculated with blood containing U.S. *A. marginale* isolates (West Coast, Oklahoma, or Virginia) and monitored for clinical signs of anaplasmosis. When the PPE was considered severe (>25%), calves were administered enrofloxacin at 12.5 mg/kg BW SC as two injections 48-hours apart (Day 0). Two additional inoculated calves were enrolled as controls. Post-treatment blood samples were collected weekly through 42 days for PPE, PCV, and cELISA inhibition testing. Both control group calves were euthanized by Day 6 for humane reasons (PCV less than 10%). In the enrofloxacin-treated calves, the mean PPE, PCV, and cELISA inhibition decreased (from Day 0 values) by Day 6. Mean PCVs generally increased (to near pre-treatment values) from Day 6 to the end of the study. The study demonstrates that administration of enrofloxacin was successful in reducing parasitemia and improving clinical signs in cattle with clinical anaplasmosis. The study is relevant as it was conducted using the intended formulation, as a challenge with U.S. isolates, and at the same dose (12.5 mg/kg BW) and route (SC) as the proposed conditional use. The difference in effectiveness between a single-dose and a two-dose regimen was not evaluated in this study, but other

evaluated sources have shown beneficial results at various doses and dosing intervals.

3. Facury-Filho, E.J., et al, 2012. Effectiveness of Enrofloxacin for the Treatment of Experimentally-Induced Bovine Anaplasmosis. *Revista Brasileira de Parasitologia Veterinária*, 21(1), 32-36.

   This study was conducted in Brazil to evaluate the effectiveness of enrofloxacin and oxytetracycline in the treatment of anaplasmosis. Twenty-four Holstein calves (approximately 3 months old) were inoculated with *A. marginale*-parasitized erythrocytes. When clinical anaplasmosis was observed (increased parasitemia [12-23% PPE] and 30% reduction of baseline PCV), calves were treated with enrofloxacin (route not specified) at 7.5 mg/kg BW once (6 calves) or at 7.5 mg/kg BW twice every three days (6 calves), oxytetracycline (6 calves), or saline (6 calves). Physical exams, blood smears, and PCV evaluations were conducted daily through 27 days post-treatment. All animals had clinical signs of anaplasmosis (such as apathy, decreased food intake, pale mucous membranes, fever, increased heart and respiratory rates, and rumen hypomotility). All of the saline-treated calves were removed for humane reasons (PPE >40% and PCV <13%) on Day 4. At two days post-treatment, both enrofloxacin-treated groups had reduced parasitemia. Body temperatures returned to normal by 4 days post-treatment, and PCVs increased and stabilized by Day 7 or Day 8 in the enrofloxacin-treated groups. There was no statistical difference in parasitemia reduction between the two enrofloxacin treatment regimens. The study demonstrates that administration of enrofloxacin at a lower dose than the proposed conditional use was successful in reducing parasitemia and improving clinical signs in cattle with clinical anaplasmosis.

The remainder of the provided information evaluated effectiveness across a variety of study designs, including differences in: dose, frequency, duration, and route of administration; animal class and age (including young calves and adult cows); infection method (natural vs. challenge); and product formulation. Despite the variation, the studies consistently showed that administration of enrofloxacin in *A. marginale*-infected cattle resulted in a decrease in parasitemia and, when evaluated, improvement in clinical variables (hematocrit and rectal temperatures). Collectively, all of the evaluated information provides reasonable expectation of effectiveness for the conditionally approved conditions of use.

III. TARGET ANIMAL SAFETY

Summary: The systemic safety of enrofloxacin when administered as a single SC injection of 12.5 mg/kg BW in beef and non-lactating dairy cattle has previously been demonstrated and is described in the FOI Summary for the original approval of Baytril® 100 (NADA 141-068) dated July 24, 1998.

Two reproductive safety studies were conducted to evaluate the safety of enrofloxacin in female cattle of reproductive age during early gestation, late gestation, and lactation. These studies showed that enrofloxacin is safe for treated cows and their offspring when administered to the cow as an intravenous (IV) injection of 15 mg/kg BW. Treatment with enrofloxacin did not affect cow health or

reproductive efficiency and injection site inflammation was the only notable test article-related finding. As described below, 15 calves died or were euthanized for *Escherichia coli*-related illness in Study No. 200290, which most likely resulted from contaminated environment or equipment. There were also three deaths in Study No. 200290 in enrofloxacin group calves caused by perforated gastrointestinal ulcers; because gastrointestinal tract ulcers occur due to a variety of causes with varying frequency in pre-weaned beef calves and the number of cases in this study is well within reported prevalence rates, it was considered unlikely that exposure to enrofloxacin was the cause.

Because the reproductive safety studies were conducted using an investigational dosage regimen, pharmacokinetic (PK) data generated from blood samples collected from lactating dairy cows administered 15 mg enrofloxacin/kg BW IV and PK data from previously conducted studies where enrofloxacin was administered by SC injection were used to confirm that the dosage regimen used in the reproductive safety studies represent a worst-case scenario in terms of drug exposure (i.e., administration of 15 mg/kg BW IV for two consecutive days results in higher exposure than the labeled Baytril® 100-CA1 dosage regimen). Because the reproductive safety studies were conducted in two different classes (one in beef cows and one in dairy cows), the PK data were also used to show that drug exposure is higher in lactating dairy cows compared to beef cattle, so reproductive safety studies conducted in dairy cows also demonstrate reproductive safety for beef cows and replacement beef and dairy heifers. Therefore, based on the reproductive safety studies and PK information summarized below, the Agency concludes that Baytril® 100-CA1 is safe when administered to female cattle of reproductive age as described in the General Information section (Section I) above. However, because residue depletion studies have not been conducted in cows producing milk for human consumption, Baytril® 100-CA1 is not approved for use in female dairy cattle 20 months of age or older, including dry dairy cows.

A. Reproductive Safety Study (Breeding/First Trimester)

Title: "Safety of Baytril® 100 (Enrofloxacin 10%) Following Intravenous Administration to Breeding (Estral) and Pregnant (1st Trimester) Beef Cows." (Study No. 200290)

Study Dates: September 2012 to August 2015

Study Location: Reedley, CA

Study Design:

Objective: To evaluate the effects of enrofloxacin on the early phases of reproduction (including follicular development, estrus, conception, placentation, initial embryonic/fetal maturation) and on the viability, growth, and health of calves born to treated cows. The study was conducted in accordance with GLP regulations (21 CFR 58).

Study Animals: A total of 450 crossbred beef cows (mostly Angus or Angus cross), between 2 and 6 years old, weighing approximately 707 - 1495 lbs. at

arrival were acquired for the study, from which 434 were subsequently enrolled. At enrollment, cows were healthy, multiparous, non-pregnant but reproductively competent. Study cows were housed in outdoor corrals and fenced pastures, were limit fed alfalfa hay, and had *ad libitum* access to trace mineral salt blocks and water. Calves remained with their dams after birth.

Experimental Design: This was a controlled, masked, reproductive safety study using a complete randomized design with no blocking factors. The study was conducted using four cohorts. Within each cohort, cows were assigned to one of five treatment groups as shown in Table III.1. below. There was a 14-day interval between the start of each cohort.

Drug Administration: Enrofloxacin injectable solution (as the U.S. commercial formulation of Baytril® 100, 100 mg enrofloxacin/mL) was used as the test article. The control group was untreated. The treatment regimens were selected based on critical development time points during the first trimester of pregnancy. Treatments were based on body weights obtained approximately 1 day prior to the first treatment and were administered by IV injection in the jugular vein, alternating sides of the neck each day. Cows were assigned to one of five treatment groups and treated as follows:

Table III.1. Treatment Groups, Study No. 200290

Group	Treatment Regimen	Number of Cows Enrolled (Bred on Day 0)	Number of Cows Pregnant (Day 60)
A	control, non-treated	87	37
B	enrofloxacin (Baytril® 100), 15 mg/kg BW by IV injection on Days -7 and -6 (folliculogenesis)	87	36
C	enrofloxacin (Baytril® 100), 15 mg/kg BW by IV injection on Days 17 and 18 (maternal recognition/early organogenesis)	86	41
D	enrofloxacin (Baytril® 100), 15 mg/kg BW by IV injection on Days 29 and 30 (early placentation/mid-organogenesis)	87	47
E	enrofloxacin (Baytril® 100), 15 mg/kg BW by IV injection on Days 44 and 45 (definitive placentation/late organogenesis)	87	35

Measurements and Observations: Animals were acclimated to the study site from 29 to 57 days prior to Day -14. Cows were synchronized using a GnRH (Days -10 and -1) and prostaglandin (Day -3) injection protocol and inseminated on Day -1 with semen from a single, purebred Angus bull. Pregnancy exams (rectal palpation) were conducted on Day 60 and Day 188; enrolled cows found to be open at the Day 60 pregnancy check were removed from the study and excluded from further reproductive safety evaluation. Physical exams were conducted on

all cows within 24 hours of arrival, on cows and calves within 24 hours of calving, and on calves at 60 days post-partum. Clinical observations were performed on study cows daily from Day -7 to approximately 14 days prior to anticipated calving, approximately every 2 hours between 6:00 AM and midnight from approximately 14 days prior to anticipated calving until all cows in the cohort had calved, and then on the cows and their calves daily from calving until the end of the study. On study days -7 to -5, 17 to 19, 29 to 31, and 44 to 46, injection site observations were recorded as part of the clinical observations. Ease of delivery and presence of dystocia were recorded for each cow at calving. Body weights were collected on study cows within 24 hours of arrival, approximately 1 day prior to the first treatment, and within 24 hours of calving, and on calves within 24 hours of birth and at 20, 40, and 60 days post-partum. A final classification of each calf as normal or abnormal was made at 60 days post-partum.

The primary safety variables were:

1. Reproductive efficiency – conception rate (percent of cows inseminated that were diagnosed as pregnant on Day 60), pregnancy attrition (percent of cows pregnant on day 60 and still pregnant on Day 188), calving rate (percent of cows reconfirmed as pregnant on Day 188 that delivered a live, full term calf), calving rate failure (percent of cows reconfirmed as pregnant on Day 188 that did not deliver a live, full term calf), abortion rate (percent of cows reconfirmed as pregnant on Day 188 that had a documented abortion), stillbirth rate (percent of cows reconfirmed as pregnant on Day 188 that resulted in a stillborn calf), and composite dystocia scores (on a scale of 0 [normal] to 4 [delivery by Caesarian section or fetotomy]).

2. Calf health – presence of congenital anomalies, body weight, and overall calf health (normal vs abnormal)

Cow health was evaluated as a secondary variable, based on clinical observations, physical exam, and body weight findings.

Statistical Methods: The experimental unit was the individual animal. For all pairwise comparisons of enrofloxacin treatment groups with the control group, a 0.10 level of significance was considered statistically significant.

Data that were binary in nature (all group rates) were analyzed using the GLIMMIX procedure assuming a binomial distribution employing a logit link. Back-transformed logits for each treatment group's percentages and 95% confidence intervals were calculated.

Data measured as categorical data were analyzed using a Chi-square analysis, testing for any significant treatment effect.

Calf body weights were analyzed using a repeated measures analysis of covariance (RMANCOVA), using the initial body weight as a covariate, with the fixed term of treatment group, and day and the interaction of treatment x day in the model. The best-fitted covariance structure was determined (smallest AIC

value) using calf ID as the subject in the repeated statement (using MIXED procedure in SAS).

Results:

The following table summarizes the cow and calf results.

Table III.2. Summary of Results, Study No. 200290

Treatment Group	Number of Cows Bred	Number of Cows Pregnant (Day 60)	Number of Cows Pregnant (Day 188)[1]	Number of Calves Born[2]	Number of Normal Calves (60 Days Post-Partum)[3]
A	87	37 (42.5%)	36 (97.3%)	36 (97.2%)	31 (86.2%)
B	87	36 (41.4%)	36 (100%)	36 (100%)	32 (88.9%)
C	86	41 (47.7%)	41 (100%)	41 (100%)	38 (92.7%)
D	87	47 (54.7%)	46 (97.9%)	44 (95.7%)	38 (86.4%)
E	87	35 (40.2%)	35 (100%)	35 (100%)	33 (94.3%)
Total	434	196	194	192	172

[1]One cow each in Groups A and D were diagnosed as non-pregnant at the Day 188 pregnancy check.
[2]In Group A, one cow had twins and one cow aborted. Two cows in Group D did not produce a calf. Twins were counted as a single birth in this calculation.
[3]Calf health is further described below.

Cow Health: A total of 434 cows were originally enrolled and bred. Of these, 237 cows were removed on Day 60 because they were determined to be non-pregnant, and one cow had an advanced pregnancy (4 - 5 months, which would have occurred prior to the study). In addition, one control group cow was removed and euthanized for lameness prior to the Day 60 exam, and one control group cow was questionable at the Day 60 pregnancy check and was confirmed non-pregnant when rechecked 14 days later. This left a total of 196 cows in the reproductive analyses (except conception rate, which was calculated based on all animals bred).

There were three cows (plus the lame cow described above) that had abnormal observations during the study. One control group cow aborted. One cow in Group D was lame but recovered without treatment and one cow in Group D had a vaginal prolapse. All of these observations were considered incidental. There were no clinically relevant differences in body weight.

Reproductive Efficiency: There were no statistically significant differences in conception rate ($p = 0.2992$), pregnancy attrition rate ($p = 0.9999$), calving rate ($p = 0.9976$), calving failure rate ($p = 1.0000$), or abortion rate ($p = 1.0000$). All reproductive efficiency variables were considered within normal ranges. There were no stillbirths and all pregnant cows delivered calves unassisted. Two cows in group D were found to be open at the expected time of parturition; these were considered within the normal range of attrition and not related to enrofloxacin treatment.

Calf Health: A total of 192 calves were born. Of these, 19 died or were euthanized prior to Day 60 post-partum, as discussed below. All calves remained in the calf parameter analyses. No calves had congenital anomalies at birth or Day 60. There was no statistical difference between treatment groups for calves classified as normal vs. abnormal ($p = 0.7143$), or for calf body weights ($p = 0.9201$).

A total of 24 calves (Group A: 4 calves, Group B: 5 calves, Group C: 5 calves, Group D: 8 calves, and Group E: 2 calves) had abnormal clinical observations and physical exam findings that were considered adverse events during the 60-day post-partum period.

Bacterial septicemia (*Escherichia coli*) was diagnosed in nine calves (Group A: 2 calves, Group B: 2 calves, Group C: 1 calf, Group D: 2 calves, and Group E: 2 calves) that died or were euthanized; four additional calves (Group B: 1 calf, Group C: 1 calf, Group D: 2 calves) received treatment for depression or diarrhea (possibly related to the *E. coli* outbreak) and recovered. One calf in Group D died due to ileocolitis of unknown etiology; two calves (one calf each in Group A and B) died or were euthanized due to pneumonia, and three calves (one calf each in Groups B, C, and D) died or were euthanized due to pulmonary edema. These calves had clinical signs of diarrhea, weakness, and/or depression, suggesting their deaths were also related to the *E. coli* outbreak. This outbreak was likely due to equipment contamination and handling of the newborn calves. Because the outbreak occurred across all treatment groups, and because *E. coli*-related illness is common in newborn calves, these adverse events were not considered to be related to enrofloxacin treatment of the pregnant cow.

One calf (Group A) died due to a traumatic injury, and one calf (Group C) developed lameness due to a foot injury and recovered following treatment; these events were incidental and not test article-related.

Three calves born to enrofloxacin-treated cows (Group C: 1 calf and Group D: 2 calves) were found dead and perforating abomasal, omasal, or jejunal ulcers with associated peritonitis were identified at necropsy. Though rare, perforating gastrointestinal ulcers have been reported in other species associated with administration of fluoroquinolones. However, gastrointestinal tract ulcers occur due to a variety of causes with varying frequency in pre-weaned beef calves and the number of cases in this study is well within reported prevalence rates. These deaths were therefore considered unlikely to be test article-related.

Conclusion: The study demonstrates the reproductive safety of enrofloxacin administered to beef cows at 15 mg/kg BW for two consecutive days at critical times during the first trimester of pregnancy, and along with the information from the PK studies described below, demonstrates the reproductive safety of Baytril® 100-CA1 in female cattle of reproductive age when used according to the approved product label.

B. Reproductive Safety Study (Late Gestation/Third Trimester)

Title: "Safety of Baytril® 100 (Enrofloxacin 10%) Following Intravenous Administration to Pregnant (3rd Trimester) Dairy Cows." (Study No. 200291)

Study Dates: September 2012 to September 2014

Study Location: Hanford, CA

Study Design:

Objective: To evaluate the effects of enrofloxacin on the late-term reproduction and gestation, fetal development, parturition, and lactation of treated cows, and the viability, growth, and health of calves born to treated cows. The study was conducted in accordance with GLP regulations (21 CFR 58).

Study Animals: A total of 60 healthy multiparous (heifers excluded) Holstein cows less than 7 years of age and weighing 1100 - 1750 lbs. were enrolled in the study. Study cows were housed on a commercial dairy and provided a total mixed ration and water *ad libitum*. Lactating cows were milked twice daily. Calves were removed from their dams following parturition, individually maintained in hutches, and provided milk replacer and calf starter rations.

Experimental Design: This was a masked reproductive safety study using a complete randomized design with no blocking factors. Sixty cows confirmed to be pregnant and in their third trimester were selected for study enrollment and randomly assigned to one of two treatment groups (enrofloxacin [Group II] or control [Group I]) of 30 cows each. The enrofloxacin-treated group was further divided into three subgroups to provide staggered treatment dates and maintain the same gestational duration at treatment (247 to 263 days).

Drug Administration: Enrofloxacin injectable solution (as the U.S. commercial formulation of Baytril® 100, 100 mg enrofloxacin/mL) was used as the test article and was administered at as a single IV injection of 15 mg/kg BW on two consecutive days. The control group was untreated. Treatments were based on body weights obtained approximately 1 day prior to the first treatment and were administered by IV injection in the jugular vein, alternating sides of the neck each day.

Measurements and Observations: To create a pool of eligible cows, all candidate cows were artificially inseminated when observed in estrus during the 39-day study breeding period with semen from a single, purebred Holstein bull. Pregnancy exams and confirmation of fetal size (via rectal palpation) were conducted at approximately 60 - 80 days, 160 – 180 days, and 212 – 252 days

post-insemination. Physical exams were conducted on cows prior to enrollment, and on calves within 24 hours of birth and at 60 days post-partum. Clinical observations were performed on study cows daily from the second pregnancy evaluation until calving and then on the calves daily from birth until the end of the study. Injection site observations were recorded at approximately 48 hours after the second treatment as part of the clinical observations. Body weights were collected on study cows approximately 1 day prior to the first treatment, and on calves within 24 hours of birth and at 20, 40, and 60 days post-partum.

Ease of delivery and presence of dystocia were recorded for each cow at calving. A final classification of each calf as normal or abnormal was made at 60 days post-partum.

The primary safety variables were:

1. Reproductive efficiency – calving rate (percent of enrolled cows that delivered a live, full term calf), calving rate failure (percent of enrolled that did not deliver a live, full term calf), abortion rate (percent of enrolled cows that had a documented abortion), stillbirth rate (percent of enrolled cows with pregnancy that resulted in a stillborn calf), and composite dystocia scores (on a scale of 0 [normal] to 4 [delivery by Caesarian section or fetotomy]).

2. Calf health – presence of congenital anomalies, body weight, and overall calf health (normal vs abnormal)

Cow health was evaluated as a secondary variable, based on clinical observations and physical exam findings.

Statistical Methods: The experimental unit was the individual animal. For all pairwise comparisons of enrofloxacin-treated group with the control group, a 0.10 level of significance was considered statistically significant.

Data that were binary in nature (all group rates) were analyzed using the GLIMMIX procedure assuming a binomial distribution employing a logit link. Back-transformed logits for each treatment group's percentages and 95% confidence intervals were calculated.

Data measured as categorical data were analyzed using a Chi-square analysis, testing for any significant treatment effect.

Calf body weights were analyzed using a repeated measures analysis of covariance (RMANCOVA), using the initial body weight as a covariate, with the fixed term of treatment group, and day and the interaction of treatment x day in the model. The best-fitted covariance structure was determined (smallest AIC value) using calf ID as the subject in the repeated statement (using MIXED procedure in SAS).

Results:

The following table summarizes the cow and calf results.

Table III.3. Summary of Results, Study No. 200291

Treatment Group	Number of Cows Enrolled	Number of Calves Born[1]	Number of Normal Calves (Day 60)[2]
I (control)	30	32	28 (87.5%)
II (enrofloxacin)	30	33	30 (90.9%)
Total	60	65	58

[1]In Group I, one cow aborted, and one calf was stillborn (no diagnosis). In Group II, one calf was stillborn (prolonged parturition/dystocia). Three cows in each treatment group had twins.
[2]Calf health is further described below.

Cow Health: One cow in the control group aborted after enrollment. All cows remained healthy and no abnormal injection site observations were recorded. All 60 enrolled cows remained in the reproductive analyses.

Reproductive Efficiency: There were no statistically significant differences in calving rate ($p = 0.5634$), calving failure rate ($p = 0.5634$), abortion rate ($p = 0.9779$), stillbirth rate ($p = 1.000$), or dystocia score ($p = 0.2829$). All reproductive efficiency variables were considered within normal ranges.

Calf Health: A total of 63 live calves were born. No calves had congenital anomalies at birth or Day 60. There was no statistical difference between treatment groups for calves classified as normal vs. abnormal ($p = 0.6603$), or for calf body weights. ($p = 0.6238$)

A total of 11 calves (5 calves in Group I and 6 calves in Group II) had abnormal clinical observations and physical exam findings that were considered adverse events during the 60-day post-partum period. In Group I (control), one calf died due to unknown cause and one calf died due to an intestinal torsion. In Group II (enrofloxacin), one calf died due to dehydration associated with an avulsion of the scapulohumeral joint (trauma-related) that was diagnosed at necropsy. None of these deaths were related to the test article. Transient mild depression was observed in 3 calves in Group I and 5 calves in Group II; all of these calves recovered following supportive therapy. No cause for the instances of depression was definitively identified but these adverse events likely resulted from stress (heat, dehorning, and/or handling). Because the depression events occurred in both treatment groups at a similar level, they were not considered related to treatment of the pregnant cow with enrofloxacin.

Conclusion: The study demonstrates the reproductive safety of enrofloxacin administered to dairy cows at 15 mg/kg BW for two consecutive days during the third trimester of pregnancy, and along with the information from the PK studies described below, demonstrates the safety of Baytril® 100-CA1 in female cattle of reproductive age when used according to the approved product label.

C. Pharmacokinetic (PK) Evaluation

Blood samples were collected for drug concentration analysis from lactating dairy cows enrolled in a margin of safety study (Study No. 200289) that were treated with 5, 10, or 15 mg enrofloxacin/kg BW IV for six consecutive days (Days 0 to 5). Blood samples were collected on Day 0 (first dose) and Day 4 (fifth dose) at pre-treatment and 15 minutes ± 30 seconds, 1 hour ± 5 minutes, 8 hours ± 15 minutes, and 24 hours ± 30 minutes post-treatment. Plasma concentrations of enrofloxacin and ciprofloxacin (an active metabolite) were measured using a validated LC-MS/MS method. The area under the plasma concentration time curve (AUC_{last}) and maximum concentration after dosing (C_{max}) were evaluated because these parameters best represent drug exposure for the safety evaluation. These parameters are summarized in Tables III.4. and III.5. below. For both enrofloxacin and ciprofloxacin, there was a dose proportional increase in C_{max} and AUC_{last} with an increase in dose and minimal accumulation with repeated doses.

Table III.4. Summary of Mean AUC_{last} (hr*µg/L) of Enrofloxacin and Ciprofloxacin after First and Fifth IV Dose in a 6 Consecutive Day Dosing Study in Lactating Dairy Cows at 5, 10 and 15 mg/kg Doses

Dose (mg/kg BW)	Dose 1 Enrofloxacin	Dose 1 Ciprofloxacin	Dose 5 Enrofloxacin	Dose 5 Ciprofloxacin
5	7721.76	3375.82	9881.94	3559.85
10	13762.46	6554.37	18359.93	6922.81
15	28651.68	10522.75	34361.45	10823.23

Table III.5. Summary of Mean C_{max} (µg/L) of Enrofloxacin and Ciprofloxacin after First and Fifth IV Dose in a 6 Consecutive Day Dosing Study in Lactating Dairy Cows at 5, 10 and 15 mg/kg Doses

Dose (mg/kg BW)	Dose 1 Enrofloxacin	Dose 1 Ciprofloxacin	Dose 5 Enrofloxacin	Dose 5 Ciprofloxacin
5	3340.29	666.29	4310.71	665.29
10	4476.00	1027.63	7628.38	1251.63
15	10974.00	1807.50	12091.88	1561.88

The PK data from lactating dairy cows in Study No. 200289 (summarized above, 15 mg/kg BW IV) and two PK studies in beef cattle (steers and heifers, Study No. 151603, 12.5 mg/kg BW IV and SC) and non-lactating dairy cows (Study No. 201098, 12.5 mg/kg BW SC) were compared to 1) show that the IV route of administration represents a worst-case scenario in terms of drug exposure, and 2) show that the data generated with lactating dairy cows support the reproductive safety in beef cows and replacement beef and dairy heifers (which are physiologically similar to beef steers and heifers).

Tables III.6. and III.7. below compare the dose normalized C_{max}, initial concentration (C_0), and the area under the concentration curve for 24 hours (AUC_{24}) for enrofloxacin and ciprofloxacin in lactating dairy cows, non-lactating

dairy cows, and beef cattle (steers and heifers). For enrofloxacin, the C_0, which represents peak exposure, was highest (1.16 µg/mL) in beef cattle. However, the dose normalized AUC_{24}, which represents overall drug exposure, was highest for both enrofloxacin and ciprofloxacin (1.91 µg*hr/mL and 0.07 µg*hr/mL, respectively) in lactating dairy cows. Further, for both enrofloxacin and ciprofloxacin, the AUC_{24} and C_{max} values for the SC route were lower than those for the IV route.

Table III.6. Summary of Mean Dose Normalized Initial Concentrations Pharmacokinetic Parameters of Enrofloxacin in Non-Lactating Dairy Cows, Lactating Dairy Cows, and Beef Steers and Heifers

Dose Normalized PK Parameter	Route	Non-Lactating Dairy	Lactating Dairy	Beef Steers and Heifers
AUC_{24} (µg*hr/mL)	IV	NA*	1.91	1.39
AUC_{24} (µg*hr/mL)	SC	1.07	NA	1.01
C_{max} (µg/mL)	IV	NA	0.73	1.06
C_{max} (µg/mL)	SC	0.07	NA	0.11
C_0 (µg/mL)	IV	NA	0.96	1.16
$T_{1/2}$ (hr)	IV	NA	2.77	6.36
$T_{1/2}$ (hr)	SC	7.95	NA	4.15
T_{max} (hr)	SC	7.33	NA	4.25

*NA, not analyzed

Table III.7. Summary of Mean Dose Normalized Pharmacokinetic Parameters of Ciprofloxacin in Non-Lactating Dairy Cows, Lactating Dairy Cows, and Beef Steers and Heifers

Dose Normalized PK Parameter	Route	Non-Lactating Dairy	Lactating Dairy	Beef Steers and Heifers
AUC_{24} (µg*hr/mL)	IV	NA*	0.7	0.68
AUC_{24} (µg*hr/mL)	SC	0.49	NA	0.54
C_{max} (µg/mL)	IV	NA	0.12	0.13
C_{max} (µg/mL)	SC	0.03	NA	0.05
C_0 (µg/mL)	IV	NA	NA	0.09
$T_{1/2}$ (hr)	IV	NA	3.6	3.48
$T_{1/2}$ (hr)	SC	9.48	NA	5.15
T_{max} (hr)	SC	8.67	NA	6.25

*NA, not analyzed

Conclusion: The PK data demonstrate that an IV dosage regimen of 15 mg/kg BW IV for two consecutive days results in higher exposure than the labeled Baytril® 100-CA1 dosage regimen of 12.5 mg/kg BW SC once. Therefore, the reproductive safety studies summarized above (Studies #200290 and 200291) demonstrate reproductive safety for the labeled regimen.

In addition, the PK data demonstrate that drug exposure is higher in lactating dairy cows compared to beef cattle. Therefore, the reproductive safety study conducted in dairy cows also demonstrates reproductive safety for beef cows and replacement beef and dairy heifers.

IV. HUMAN FOOD SAFETY

A. Microbial Food Safety

Background and Outcome of Risk Assessment

The Agency evaluated microbial food safety information for enrofloxacin for the treatment of clinical anaplasmosis in cattle, specifically in steers, beef heifers, bulls intended for slaughter, replacement dairy heifers (up to 20 months of age), beef cows, replacement beef bulls, and replacement dairy bulls, classes which encompass the classes on the conditionally approved labeling. For this conditional approval, the sponsor provided information to the Agency in the form of a microbial food safety hazard characterization. The hazard to human health is defined as a human illness caused by fluoroquinolone-resistant foodborne bacteria (*Campylobacter* spp., *Salmonella* spp., and multidrug resistant (MDR) *Salmonella* spp.) attributable to consumption of contaminated beef originating from cattle treated with enrofloxacin for clinical anaplasmosis, and subsequently treated with a human antibiotic from the fluoroquinolone class of antimicrobials. The hazard characterization included information to describe the extent of use of enrofloxacin in cattle as a result of the addition of this new claim, and included information to describe the use of enrofloxacin as a single injection at 12.5 mg/kg BW in cattle and its impact on the emergence of resistant bacteria or resistance determinants among bacteria of public health concern in or on treated cattle under proposed conditions of use. The Agency evaluated the information submitted by the sponsor, and considered current fluoroquinolone and quinolone susceptibility profiles of *Salmonella* spp., *Escherichia coli*, and *Campylobacter* spp., including their prevalence in the food commodity of concern (retail beef) and target animal (cattle).

The hazard characterization demonstrated 1) that the addition of this supplemental use in cattle will only result in a minor increase in enrofloxacin use over current uses for bovine respiratory disease (BRD), and 2) that the conditions of use, including appropriate use parameters to determine if cattle are eligible to receive enrofloxacin for the treatment of clinical anaplasmosis, allowed the Agency to conclude that antimicrobial resistance concerns for fluoroquinolone-resistant *Campylobacter* and *Salmonella* originating from treated cattle are minimized.

Based upon this evaluation and the following antimicrobial resistance risk mitigating factors:

- Enrofloxacin will be a prescription (Rx) only drug;
- Treatment will be via a single dose administration of enrofloxacin at 12.5 mg/kg BW administered once to cattle exhibiting clinical signs of anaplasmosis;

- Extra-label use of fluoroquinolones is prohibited by law (21 CFR 530.21) in food-producing animals;
- Susceptibility to fluoroquinolones is currently monitored by the National Antimicrobial Resistance Monitoring System (NARMS);

the Agency concludes that the use of enrofloxacin in cattle for the treatment of anaplasmosis will not result in a significant risk to public health with respect to development of fluoroquinolone resistance among foodborne *Campylobacter* and *Salmonella* originating from enrofloxacin-treated cattle.

Decision Statement:

The impact of this supplemental use of enrofloxacin in cattle for the treatment of anaplasmosis on microbial food safety was carefully considered by the Agency. For this conditional approval, the sponsor provided information to the Agency through a microbial food safety hazard characterization. The sponsor identified the hazard of concern as human illness caused by fluoroquinolone-resistant foodborne bacteria (*Campylobacter* spp., *Salmonella* spp., or MDR *Salmonella* spp.). The hazard characterization demonstrated 1) that this additional use of enrofloxacin in cattle will only result in a minor increase in enrofloxacin use over its current uses for BRD, and 2) that the conditions of use, including appropriate use parameters to determine if cattle are eligible to receive enrofloxacin for the treatment of anaplasmosis, allowed the Agency to conclude that antimicrobial resistance concerns for fluoroquinolone-resistant *Campylobacter* and *Salmonella* originating from treated cattle are minimized.

B. Toxicology

Reassessment of the toxicological acceptable daily intake (ADI) was not needed for this conditional approval. The Freedom of Information (FOI) Summaries for the original approval of NADA 141-068, dated July 24, 1998, and a supplemental approval, dated February 13, 2008, contain a summary of all toxicology studies and information.

CVM did not require additional information for the effect of enrofloxacin residues on human intestinal flora for this conditional approval. The FOI Summaries for supplemental approvals of NADA 141-068 dated February 13, 2008; July 24, 2012; and December 23, 2014, contain summaries of all information used to assess the effect of residues on human intestinal flora.

C. Establishment of the Final ADI

The final ADI is the toxicological ADI of 3 µg/kg bw/day for total residues of enrofloxacin derived from a 3-month subchronic oral toxicity study in dogs. The codified ADI is listed under 21 CFR 556.226.

D. Safe Concentrations for Total Residues in Edible Tissues

Reassessment of the safe concentrations for total residues of enrofloxacin were not needed for this approval. The safe concentrations of total residues of enrofloxacin in individual edible tissues of cattle are 0.6 ppm for muscle, 1.8 ppm for liver, 3.6 ppm for kidney, 3.6 ppm for fat, and 6 ppm for the injection sites.

E. Residue Chemistry

CVM did not require residue chemistry studies for this conditional approval. The FOI Summary for the original approval of NADA 141-068 dated July 24, 1998, contains a summary of residue chemistry studies for cattle.

F. Analytical Method for Residues

The FOI Summary for the original approval of NADA 141-068 dated July 24, 1998, contains the analytical method summaries for enrofloxacin in cattle.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to Baytril® 100-CA1:

Not for use in humans. Keep out of reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight.

VI. AGENCY CONCLUSIONS

The data submitted in support of this application satisfy the requirements of section 571(b) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). The data demonstrate that Baytril® 100-CA1, when used according to the label, is safe and has a reasonable expectation of effectiveness for the treatment of clinical anaplasmosis associated with *Anaplasma marginale* in replacement dairy heifers under 20 months of age and all classes of beef cattle except beef calves less than 2 months of age and beef bulls intended for breeding (any age); not for use in any other class of dairy cattle or in veal calves. Additionally, data demonstrate that residues in food products derived from species treated with Baytril® 100-CA1 will not represent a public health concern when the product is used according to the label.

A. Conditional Approval Eligibility

Conditional approval is an option for animal drugs intended for use in "minor species" (all animals other than horses, cattle, pigs, chickens, turkeys, dogs, and cats) or for a "minor use" (a disease or condition affecting a limited population) in one of the major species. Eligibility for conditional approval under a minor use status requires a justification that the disease or condition for which the proposed product is intended affects a "small number of animals". The Agency's current "small number of animals" threshold for cattle is 310,000 (21 CFR 516.3).

The sponsor used the information listed below to generate an estimate of the number of adult cattle (beef and dairy) with clinical cases of anaplasmosis per year.

- Estimates of beef and dairy cattle annual populations from United States Department of Agriculture (USDA) reports.[2]
- Estimates of annual overall mortality rates among adult beef and dairy cattle from USDA publications.[3,4]
- Estimates of the percentage of annual mortality associated with "other known diseases" (including anaplasmosis) from USDA publications.[5,6,7]
- An estimate of the mortality rate among adult cattle in the U.S. experiencing clinical anaplasmosis from two published articles on anaplasmosis.[8,9]

Using a mathematical model, the sponsor calculated that clinical anaplasmosis affects, a maximum of 251,045 adult cattle annually in the U.S.

Based on a review of the available information, the Agency made modifications to the estimates based on the following assumptions:

- the rate of occurrence of clinical anaplasmosis is similar between dairy and beef cattle, and
- the mortality rate from anaplasmosis increases with age in cattle and dairy cattle are generally maintained to an older age than beef cattle.

The revised estimates, while accounting for uncertainties from this assessment, were as follows:

- best-case scenario: 49,500 animals with clinical anaplasmosis on an annual basis, and
- worst-case scenario: 240,900 animals with clinical anaplasmosis on an annual basis.

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[2] USDA National Agriculture Statistics Service website (https://www.nass.usda.gov/)

[3] USDA:APHIS:VS:CEAH. "Beef 2007-08, Part IV: Reference of Beef Cow-calf Management Practices in the United States, 2007-08", #523.0210. February 2010.

[4] USDA-APHIS-VS-CEAH-NAHMS. "Dairy 2014, Dairy Cattle Management Practices in the United States, 2014", #692.0216. February 2016.

[5] USDA:APHIS:VS:CEAH. "Mortality of Calves and Cattle on U.S. Beef Cow-calf Operations", #568.0510. May 2010.

[6] USDA:APHIS:VS:CEAH. "Beef 2007-08, Part 1: Reference of Beef Cow-calf Management Practices in the United States, 2007-08", #N512-1008. October 2008.

[7] USDA:APHIS:VS:CEAH. "Dairy 2007, Part I: Reference of Dairy Cattle Health and Management Practices in the United States, 2007", #N480.1007. October 2007.

[8] Kocan KM, de la Fuente J, Blouin EF, et al. The natural history of *Anaplasma marginale*. 2010;167(2-4):95-107.

[9] Richey EJ. Bovine anaplasmosis. Proceedings of the 24th Annual Conference of the American Association of Bovine Practitioners, Orlando, FL, 3-11. 1991.

By all estimates performed, the number of cattle with clinical anaplasmosis on an annual basis is lower than 310,000. Therefore, the Agency concluded that the use of enrofloxacin to treat clinical anaplasmosis in cattle in the U.S. constitutes a minor use, and the product and indication are eligible for conditional approval.

B. Marketing Status

Baytril® 100-CA1 is conditionally approved for one year from the date of approval and is annually renewable for up to four additional one-year terms.

This product may be dispensed only by or on the order of a licensed veterinarian (Rx marketing status). This decision was based on the following factors: adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this drug product, and because restricting this drug product to use by or on the order of a licensed veterinarian is critical for assuring the safe and appropriate use of this drug product in animals in order to slow or prevent any potential for the development of bacterial resistance to antimicrobial drugs.

C. Exclusive Marketing Rights

Baytril® 100-CA1, as conditionally approved in our conditional approval letter, qualifies for SEVEN years exclusive marketing rights beginning as of the date of our approval letter. This drug qualifies for exclusive marketing rights under section 573(c) of the FD&C Act because it is a designated new animal drug under section 573(a) of the act. Except as provided in section 573(c)(2) of the FD&C Act, the Agency may not approve or conditionally approve another application submitted for the same drug in the same dosage form with the same intended use as Baytril® 100-CA1. Exclusive marketing rights begin as of the date of our conditional approval letter.

D. Patent Information

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.

VII. APPENDIX

The Agency Conclusions section of the FOI Summary was revised to include information about the minor use determination made by the Agency and to provide corrected language for the Exclusive Marketing Rights subsection. In addition, sponsor references were updated to reflect the August 10, 2020, transfer of ownership of application number 141-527 to Elanco US Inc.