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Antimicrobial-resistant *Salmonella* Serotypes in Feces of Bison in North Dakota

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Abstract

The objective of this study was to determine the occurrence of *Campylobacter, Salmonella* and *Escherichia coli* O157:H7 in fecal samples from a herd of bison and determine antimicrobial susceptibility patterns of pathogens isolated. Fecal grabs were obtained from the rectum of each bison (n=20). None of the fecal samples tested positive for *E. coli* O157:H7 or *Campylobacter* spp. Two of 20 (10%) bison fecal samples tested positive for *Salmonella* spp. The *Salmonella* isolates belonged to the serotypes *Salmonella* Typhimurium (Copenhagen) and *Salmonella* Worthington. Both isolates were resistant to the same 13 of 20 antimicrobials tested, including macrolides (erythromycin, tilmicosin, tylosin), tetracyclines (chlortetracycline, oxytetracycline), florfenicol, most sulfonamides and penicillin. *S.* Worthington was also resistant to ampicillin. They were susceptible to at least six antimicrobials including ceftiofur, enrofloxacin and some aminoglycosides.

Keywords: bison, salmonella, campylobacter, *E. coli* O157:H7, MAP

Résumé

L'objectif de cette étude était de déterminer la présence de Campylobacter, de Salmonella et d'Escherichia coli O157:H7 dans des échantillons fécaux provenant d'un troupeau de bison et d'identifier le profil de résistance aux antimicrobiens des pathogènes isolés. Les échantillons fécaux ont été obtenus à partir du rectum de chaque bison (n=20). Aucun des tests des échantillons fécaux n'était positif pour E. coli O157:H7 ou pour Campylobacter. Deux des 20 échantillons fécaux de bison (10%) étaient positifs pour Salmonella spp. Les isolats de Salmonella appartenaient aux sérotypes Salmonella typhimurium (Copenhagen) et Salmonella worthington. Les deux isolats étaient résistants à 13 des mêmes antimicrobiens parmi les 20 testés incluant des macrolides (érythromycine, tilmicosine, tylosine), des tétracyclines (chlortétracycline, oxytétracycline), le florfénicol, la plupart des sulphonamides et la pénicilline. L'isolat S. worthington était aussi résistant à l'ampicilline. Les isolats étaient aussi susceptibles à au moins six antimicrobiens incluant la ceftiofur, l'enrofloxacine et certains aminoglycosides.

Introduction

Campylobacter, pathogenic *Escherichia coli* (*E. coli*) and *Salmonella* are among the major bacterial foodborne pathogens both in the United States (US)[5,21] and worldwide.[18,20] In 2005, the Foodborne Diseases Active Surveillance Network (FoodNet) of the US Centers for Disease Control and Prevention (CDC) reported a total of 16,614 laboratory-confirmed cases of infections, and overall incidence per 100,000 population was 14.55 for *Salmonella*, 12.72 for *Campylobacter* and 1.06 for shigatoxin-producing *E. coli* - STEC O157.[5] Although a range of animal hosts, including cattle, have been identified as reservoirs for *Salmonella*,[8,9,10,27] *Campylobacter*[3,9,22] and *E. coli* O157:H7,[14] data on prevalence of these major bacterial pathogens in bison is scanty.

The emergence of resistant and multi-resistant bacteria has become an important worldwide sanitary problem, impacting both veterinary medicine and public health through the potential for therapeutic failures.[16] Antimicrobial resistance among bacterial isolates from animals is of concern because of the potential for these organisms to be foodborne or zoonotic pathogens, or to be donors of resistance genes to human pathogens.[16] Recent investigations suggest the environment, including water supplies and animal feed,[26] as well as cattle,[9] pigs,[10] and other farm animals and poultry,[19] may play an important role in human infection with these pathogens.

American bison (*Bison bison*) production has traditionally been free-range, presumptively without use of antimicrobials.[24] The possibility of cross transmission of several bovine pathogens between free-ranging bison and domestic cattle has been suggested,[15] but not well investigated. Taylor *et al*[24] evaluated 101 free-ranging American bison from Yellowstone National

Park in Wyoming for serologic exposure to infectious organisms that commonly infect cattle. No titers were detected for bluetongue virus, bovine leukemia virus, or *Campylobacter fetus*. Detectable antibodies were present against *Anaplasma marginale* (eight of 76, 11%), bovine respiratory syncytial virus (31 of 101, 31%), bovine viral diarrhea virus (31 of 101, 31%), bovine herpesvirus 1 (29 of 76, 38%), parainfluenza-3 virus (27 of 75, 36%), *Leptospira interrogans* Icterohaemorrhagiae (four of 101, 4%), *L. interrogans* Hardjo (seven of 101, 7%), *L. interrogans* Autumnalis (one of 101, 1%), *L. interrogans* Bratislava (seven of 101, 7%) and *L. interrogans* Australis (one of 101, 1%). Low antibody titers and the lack of clinical signs suggest that while previous exposure to infectious organisms may have occurred, none appeared to have active infections. The objective of this study was to determine the presence of *Salmonella* spp, *Campylobacter* spp and *E. coli* O157: H7 in fecal samples from an apparently healthy bison herd in North Dakota.

Materials and Methods

Study Herd

Samples were collected on June 28, 2005 from bison in a herd located in southeastern North Dakota. These bison were purchased about one year earlier from a ranch in central North Dakota. Of 21 bison purchased, 17 were adults and four were calves, with age and gender distribution of the 20 surviving animals shown in Table 1. Prior to our visit, one bison died on pasture and no postmortem examination was performed. The bison grazed together on a 22-acre pasture with a ground well as the water source. Average temperature and rainfall for the month of sampling were: high = 75.0°F (23.9°C); low = 57.7°F (14.3°C); total rainfall = 18.54 inches (216.8 mm) (North Dakota Agricultural Weather Network [NDAWN] - available at http://ndawn.ndsu.nodak.edu/). No other animals were raised on the farm with the bison. Although the animals were not housed, a pole barn was available but they seldom occupied it. The pasture was incompletely fenced, making it possible for wildlife and birds to access the animals. No antibiotic use in the animals was reported.

All animals were simultaneously tested for *Neospora caninum* antibodies using the *Neospora caninum* Antibody Test Kit,[a] bovine viral diarrhea virus (BVDV) using the BVDV Antigen Test Kit[b] and *Mycobacterium avium* subsp *paratuberculosis* (Johne's disease). All bison were negative to the three tests except for one bull that tested positive for *Neospora caninum* antibodies. None of these tests were validated for use in bison.

Sampling Procedure

A total of 20 bison remained in the herd on the date samples were collected. Animals were run through a chute, and approximately 20 grams of feces were collected from the rectum of each animal using a clean glove to collect each sample. Samples were transferred into sterile plastic cups, placed on ice and transported within two hours to the laboratory in the Department

Table 1. Characteristics of bison herd sampled by age, gender and *Salmonella* status.

Animal ID	Age	Gender	Age	*Salmonella* status
1	>1 year	Female	Adult	Negative
2	>1 year	Female	Adult	Negative
4	>1 year	Female	Adult	Negative
15	>1 year	Female	Adult	Negative
16	>1 year	Female	Adult	Negative
17	>1 year	Female	Adult	Negative
18	>1 year	Female	Adult	*S.* Worthington
19	>1 year	Female	Adult	Negative
20	>1 year	Female	Adult	Negative
21	>1 year	Female	Adult	Negative
22	>1 year	Female	Adult	Negative
23	>1 year	Female	Adult	Negative
24	>1 year	Female	Adult	*S.* Typhimurium Copenhagen
51	1 year	Male	Bull	Negative
52	1 year	Female	Calf	Negative
53	1 year	Female	Calf	Negative
54	1 year	Female	Calf	Negative
55	1 year	Female	Calf	Negative
56	1 year	Male	Bull	Negative
97	>1 year	Male	Bull	Negative

of Veterinary and Microbiological Sciences at North Dakota State University for culture.

Laboratory Procedures

Fecal samples were cultured using methods optimized for the detection of *Salmonella* spp in bovine feces.[13] Briefly, fecal specimens were enriched overnight followed by incubation with immunomagnetic beads specific for *Salmonella*[c] according to the manufacturer's directions. After the final wash, the beads were transferred to 10 mL of Rappaport Vassiliadis R10 (RV) broth[d] and incubated at 107.6°F (42°C) for 24 hours. Following incubation, RV cultures were streaked onto modified brilliant green agar[e] and mannitol lysine crystal violet brilliant green agar.[f] Colonies with typical *Salmonella* characteristics were stabbed in 10-mL agar slants of lysine iron agar[e] and triple sugar iron agar,[e] and biochemical results were read after 24 hours incubation. All samples were then plated on media selective for *Salmonella*. Presumptive positive isolates were sent for serotyping to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa.

Optimized methods for culturing and detecting *E. coli* O157:H7 from fecal samples were used.[12,14] Briefly, sub-samples (about 10 grams) of the fecal samples were put into 90 mL of enrichment broth in whirl-pak bags[g] and incubated at 98.6°F (37°C) for six hours. The enrichment broth was then subsequently subjected to biochemical tests as previously described[12,14] including immunomagnetic separation, and further identified as O157:H7 by latex agglutination.[h]

For *Campylobacter* spp, each sample was streaked onto campy-cefex media. The plates were incubated under a microaerophilic atmosphere in an anaerobic chamber using a microaerophilic gas generating system[i] at 107.6°F for 48 hours. Typical colonies (pink with clear to diffuse growth) were transferred onto Mueller Hinton agar[e] and reincubated under the same conditions. Presumptive *Campylobacter* isolates were sent to the US Department of Agriculture (USDA), National Animal Disease Center (NADC), Ames, IA, for speciation.

Mycobacterium avium subspecies *paratuberculosis* testing was by conventional fecal culture on Herrold's egg yolk (HEY) agar slants performed as previously described,[28] with brief modifications. Briefly, one gram of feces was added to 20 mL of sterile distilled water, tubes were shaken for 30 minutes and then allowed to stand undisturbed for 30 minutes. Five mL of supernatant were added to a decontaminant mixture containing 25 mL of 0.9% hexadecylpyridinium chloride monohydrate[j] (HPC) and 30 mL of brain heart infusion (BHI) broth, and the samples were allowed to decontaminate overnight at 98.6°F (37°C). Samples were centrifuged at 900 xg for 30 minutes and the supernatant discarded. Pellets were re-suspended in 1 mL of antibiotic brew containing 50% BHI broth with 100 µg/mL vancomycin, 100 µg/mL nalidixic acid, and 50 µg/mL amphotericin B. Tubes were vortexed and incubated overnight at 98.6°F. HEY slopes were inoculated with 0.25 mL of the suspension. Each sample was cultivated in duplicate on HEY with mycobactin J and one tube without mycobactin as the culture medium. Tubes were incubated at 98.6°F and observed at two-week intervals for 16 weeks. Isolation of a slow-growing, acid-fast organism with colonial morphology typical of MAP on HEY with mycobactin, but not on HEY without mycobactin, was considered a positive culture.

Antimicrobial Resistance

Antimicrobial resistance of *Salmonella* isolates was determined using a panel of antimicrobials according to the manufacturer's instructions.[k] Each isolate was screened for resistance using a CMV1ABPF large animal plate, using full-range minimum inhibitory concentration. Antimicrobials tested were ampicillin, apramycin, ceftiofur, chlortetracycline, clindamycin, enrofloxacin, erythromycin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulfachlorpyridazine, sulfadimethoxine, sulfathiazole, tiamulin, tilmicosin, trimethoprim/sulfamethoxazole and tylosin.

Results

None of the fecal samples tested positive for *E. coli* O157:H7 or *Campylobacter* spp. Two of 20 (10%) fecal samples were positive for *Salmonella*. The *Salmonella* isolates belonged to serotypes *Salmonella* Typhimurium (Copenhagen) and *Salmonella* Worthington. In a panel of 20 antimicrobials, *Salmonella* Typhimurium (Copenhagen) was resistant to 13 of 20 antimicrobials (65% resistance), including macrolides (erythromycin, tilmicosin, tylosin), tetracyclines (chlortetracycline, oxytetracycline), florfenicol, most sulfonamides, and penicillin, and susceptible to seven antimicrobials including ceftiofur, enrofloxacin, some aminoglycosides and ampicillin (Table 2). *Salmonella* Worthington was resistant to 14 of 20 antimicrobials (70% resistance), including macrolides (erythromycin, tilmicosin, tylosin), tetracyclines (chlortetracycline, oxytetracycline), florfenicol, some sulfonamides, and penicillins (penicillin and ampicillin), and susceptible to six antimicrobials including ceftiofur, enrofloxacin and some aminoglycosides (Table 2). Except for ampicillin, both *Salmonella* isolates were resistant to the same antimicrobials (Table 3).

Discussion

Given the limited number of bison sampled, the *Salmonella* shedding point prevalence of 10% in this

bison herd was similar to that reported in beef cattle[3,11] and other livestock[4] in the US. A cross-sectional study of 212 cattle from seven cow-calf operations in North Dakota reported a *Salmonella* spp shedding point prevalence of 7% (15 of 212).[25] In another study[4] that assessed *Salmonella* spp presence in white-tailed deer (*Odocoileus virginianus*) and domestic livestock simultaneously grazing the same rangeland in the month of September, researchers reported *Salmonella* prevalence of 7.69% (2/26) and 7.32% (6/82) in deer and

Table 2. Antibiotic sensitivity and resistance of *Salmonella* isolates from a bison herd.

Antibiotics	*Salmonella* isolates	
	S. Typhimurium (Copenhagen)	*S.* Worthington
Aminoglycosides		
Apramycin	S	S
Gentamycin	S	S
Neomycin	S	S
Spectinomycin	R	R
Sulfanamides/Potentiated Sulfonamides		
Trimethoprim/Sulfamethoxazole	S	S
Sulfadimethoxine	R	R
Sulfachlorpyridazine	R	R
Sulfathiazole	R	R
Cephalosporins		
Ceftiofur	S	S
Quinolones/Fluoroquinolones		
Enrofloxacin	S	S
Pleuromutilins		
Tiamulin	R	R
Chloramphenicol Analog		
Florfenicol	R	R
Penicillins		
Ampicillin	S	R
Penicillin	R	R
Tetracyclines		
Chlortetracycline	R	R
Oxytetracycline	R	R
Macrolides		
Erythromycin	R	R
Tilmicosin	R	R
Tylosin (tartrate base)	R	R
Misc.		
Clindamycin	R	R

R= Resistant, S= Susceptible, I-Intermediate

Table 3. Antibiotic resistance patterns of *Salmonella* isolates from bison.

Isolate	Resistance pattern
S. Typhimurium (Copenhagen)	Chlo-Oxy-Spec-Sulfachlo-Sulfadi-Sulfathi
S. Worthington	Amp-Chlo-Oxy-Spec-Sulfachlo-Sulphadi-Sulfathi

Chlo=Chlortetracycline, Oxy=Oxytetracycline, Amp=Ampicillin, Spec=Spectinomycin, Amp=Ampicillin, Sulfachlo=Sulfachlorpyridazine, Sulfadi=Sulfadimethoxime

sheep, respectively, and a lower prevalence of 3.70% (3/81) and 1.25% (1/80) in goats and cattle, respectively.

Dargatz *et al*[7] evaluated the presence of *Salmonella* in fecal samples from cattle in US feedlots (73 feedlots in 12 states during October 1999 to September 2000). Fecal samples were not collected from individual animals, instead, fecal samples were collected from pen floors for culture. Pens of cattle selected for sampling were those with cattle that had been in the feedlot for the shortest period of time, the longest period of time and a randomly selected pen from the remaining pens. Overall, 6.3% (654/10,417) of samples cultured positive for *Salmonella* spp; 22.2% (94/422) of pens and 50.7% (37/73) of feedlots had one or more positive samples. There was little difference in the proportion of positive samples between short-fed (6.1%, 212/3482), random (6.4%, 217/3400) and long-fed (6.4%, 224/3485) pens of cattle. Culture results from pens of cattle in the feedlot the shortest period of time may be most reflective of *Salmonella* prevalence on pasture, where the environment is more similar to that grazed by the bison in our study. This speculation must be approached with caution, however, as the feedlot cattle could have been exposed during marketing, transport, or after feedlot entry.

In a large study[3] conducted to assess risk factors associated with hide and carcass contamination of beef cattle during transport to slaughter, a total of 281 salmonellae were isolated from 1,050 rectal (400), hide (400), carcass (200) and environmental (50) samples. For feedlot cattle, salmonellae were recovered from 4.0% of rectal samples, 37.5% of hide samples, 19.0% of carcass samples and 47.4% of environmental samples. In non-feedlot cattle, salmonellae were recovered from 10.9% of rectal samples, 37.5% of hide samples, 54.2% of carcass samples and 50.0% of environmental samples[3] while grazing on pasture. This ubiquitous nature of *Salmonella* further confirms our reluctance to speculate the prevalence of *Salmonella* in feeder steers and heifers prior to feedlot entry.

It is possible that the time of sampling may influence the prevalence of *Salmonella* reported, as seasonal changes have been reported to affect prevalence of *Salmonella* fecal shedding in cattle. Dargatz *et al*[7] reported samples collected during the period of April to June (6.8%; 209/3054) and July to September (11.4%; 286/2500) were more likely to be positive than those collected during October to December (4.0%; 73/1838) and January to March (2.8%; 86/3025). In this study we sampled bison in June 2005, while Theis[25] sampled cattle from September to November, 2004.

The *Salmonella* isolated in this study belonged to the serotypes *Salmonella* Typhimurium (Copenhagen) and *Salmonella* Worthington. Bovine are a common source of *Salmonella* Typhimurium.[6] It is interesting to note that the same serotypes, *Salmonella* Typhimurium (Copenhagen) and *Salmonella* Worthington, were recovered from cattle on cow-calf operations in North Dakota during the same year.[25] However, Beach *et al*[2] reported that the five serotypes most commonly associated with feedlot cattle and their environment were *Salmonella* Anatum (18.3% of isolates), *Salmonella* Kentucky (17.5%), *Salmonella* Montevideo (9.2%), *Salmonella* Senftenberg (8.3%) and *Salmonella* Mbandaka (7.5%). The five serotypes most commonly associated with non-feedlot cattle and their environment were *Salmonella* Kentucky (35.4%), *Salmonella* Montevideo (21.7%), *Salmonella* Cerro (7.5%), *Salmonella* Anatum (6.8%) and *Salmonella* Mbandaka (5.0%).[2]

In this study, both *Salmonella* isolates were susceptible to at least six antimicrobials on the panel including ceftiofur and enrofloxacin, both of which are clinically important to beef and dairy practitioners. Both isolates demonstrated multidrug resistance (resistance to ≥ 13 antimicrobials) in a panel of 20 antimicrobials, with resistance most frequently to tetracycline, streptomycin and/or ampicillin. Dargatz *et al*[7] determined antimicrobial resistance patterns of *Salmonella* spp recovered from feedlot cattle using a panel of 17 antimicrobials. The majority of isolates (62.8%, 441/702) were sensitive to all of the antimicrobials tested. Resistance was most frequently observed to tetracycline (35.9%, 252/702) followed by streptomycin (11.1%, 78/702), ampicillin (10.4%, 73/702) and chloramphenicol (10.4%, 73/702). Resistance to two or more antimicrobials (multidrug resistance) was observed for 11.7% (82/702) of isolates.

It is also noteworthy that *Salmonella enterica* serovar Hadar was the major *Salmonella* serotype isolated from processed bison carcasses originating in the same region as our sampled animals.[17] These salmonellae organisms were resistant to tetracycline, gentamicin, sulfamethoxazole and streptomycin,[17] similar to results from isolates in apparently healthy bison in our study. In the absence of studies that correlate recovery of *Salmonella* from the same bison pre and post-harvest, it is difficult to ascertain the sources of contamination of bison carcasses post-harvest.

In comparison with human isolates, 2,613 isolates were tested in 1999-2000 at the 17 public health laboratories participating in National Antimicrobial Resistance Monitoring System (NARMS). Of these, 26% (679) were resistant to more than one antimicrobial compound, and 21% (546) were multidrug resistant (resistant to >2 agents).[1] Three multidrug resistant strains accounted for 10% (263/2613) of all *Salmonella* isolates, 38% (263/679) of resistant isolates and 48% (263/546) of multidrug resistant isolates. In particular, 30% (162/546) of multidrug resistant *Salmonella* were

S. Typhimurium R-type ACSSuT, 12% (63/546) were *S.* Typhimurium R-type AKSSuT and 7% (38/546) were *S.* Newport R-type ACSSuT; no other multidrug resistant patterns accounted for more than 5% of multidrug resistant salmonellae.

In spite of reports that antibiotics were not used in the study herd, and that no other domestic animals were raised on the farm with the bison, antimicrobial resistance was detected in the *Salmonella* isolates recovered. It is possible that wildlife, birds and other domestic livestock had access to the animals and that *Salmonella* isolated from the bison could have acquired resistance through horizontal transfer from other multidrug resistant organisms originating from wildlife, birds or other domestic livestock.

None of the fecal samples tested positive for *E. coli* O157:H7. The transient[23] and seasonal[11] nature of shedding of these organisms, and the low prevalence of this organism would make detection unlikely in a pool of 20 animals. Previous researchers reported very low prevalence of *E. coli* O157:H7 (1.3%) in cow-calf operations[23] and white-tailed deer grazing the same rangeland with livestock.[4] In the latter study,[4] *E. coli* O157 was found in fecal samples from 1.25% of cattle and 1.22% of sheep sampled in September, however, no *E. coli* O157 was found in other sampled months or in goats or white-tailed deer. Failure to detect *E. coli* O157:H7 in bison feces in this study does not indicate absence of the organism. Future studies on more bison herds utilizing longitudinal study designs are needed to contribute to our understanding of the ecology of this organism in bison.

No fecal samples in this study tested positive for *Campylobacter* spp. A previous study[24] that evaluated 101 free-ranging American bison from Yellowstone National Park, Wyoming for exposure to infectious organisms reported no antibody titers for *Campylobacter fetus*. Failure to detect *Campylobacter* spp in bison feces in this study does not necessarily indicate absence of these organisms. Additional studies and diagnostic tests validated for bison are needed to better understand the ecology of these organisms in bison.

Conclusion

These data indicate that salmonellae were shed in feces of bison in this North Dakota herd in a prevalence range similar to that of cattle herds in the US. However, the point prevalence of salmonellae in this study must be interpreted with caution because of the small herd/sample size. Recovered isolates were multidrug resistant, and highlight that antimicrobial resistant *Salmonella* organisms are present in bison. The multidrug resistance reported among the *Salmonella* isolates warrants further study considering that serotype *S.* Typhimurium is widely distributed and has the potential to impact human and animal health.

Endnotes

[a]IDEXX Laboratories, Inc, 1 IDEXX Drive, Westbrook, ME
[b]Syracuse Bioanalytical, Inc, 23 Corporate Circle, East Syracuse, NY 13057
[c]Dynabeads® anti-*Salmonella*, Dynal Biotech, Inc, Lake Success, NY
[d]Becton Dickinson, Sparks, MD
[e]Becton Dickinson, Sparks, MD
[f]Oxoid LTD, Basingstoke, UK
[g]Nasco, Fort Atkinson, WI
[h]Remel, Lenexa, KS
[i]AnaeroPack System™, Mitsubishi Gas Chemical America, Inc, New York, NY
[j]Sigma, USA, St. Louis, MO 63103
[k]Sensititre®, Trek Diagnostics System, Inc, Westlake, OH

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Baytril® 100 (enrofloxacin) is approved for use in dairy replacement heifers less than 20 months of age!

IT'S ABOUT TIME.

Injectable
Baytril® 100
(enrofloxacin)
Right the first time®

BAYER

IT'S ABOUT TIME.

It's about time dairy-heifer producers count on the same performance beef-cattle producers trust to fight bovine respiratory disease (BRD). For years, Baytril® 100 (enrofloxacin) Injectable Solution has proven to quickly kill the bacteria that cause BRD. That performance is now approved for dairy replacement heifers less than 20 months of age.

It's about time to recognize the impact of BRD in dairy replacement heifers. When BRD hits a dairy replacement heifer, it attacks fast and hard. It will rob a calf of healthy lung tissue each day it goes untreated and lung damage can be permanent. It jeopardizes not only the health of the calf, but also the earning potential of your investment.

Baytril® 100 (enrofloxacin) goes directly to the site of the infection and starts killing BRD-causing bacteria in minutes, not days.[1]

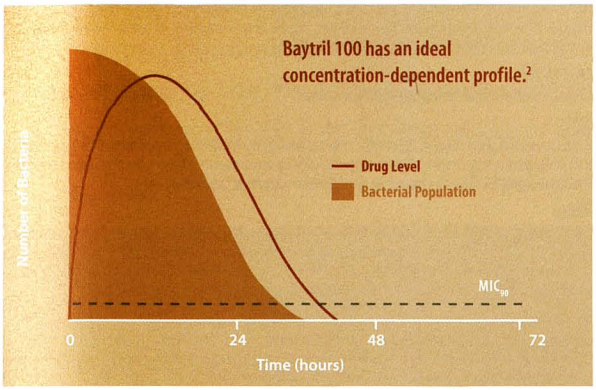
Dairy replacement heifers are not just a financial investment: they represent the future of your operation. They're too valuable to risk, especially to the damage BRD can inflict.

- The cost of replacement heifers is second only to feed costs in most dairy operations.
- The cost of replacement heifers has increased considerably in the last few years, reaching approximately $2,000 in summer 2007.

Federal law restricts this drug to use by or on the order of a licensed veterinarian. Extra-label use of this product in food-producing animals is prohibited.

Because BRD moves fast, you need an antibiotic that works fast.

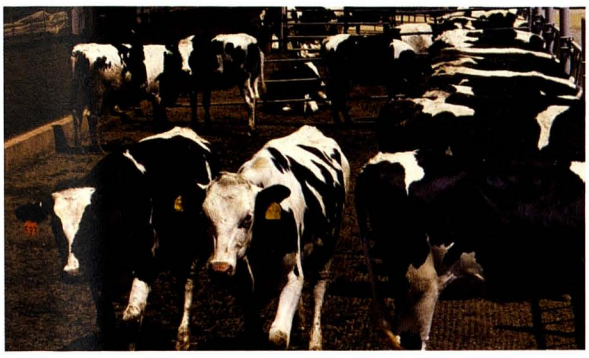
Baytril® 100 (enrofloxacin): concentration-dependent to start working in minutes.[2]



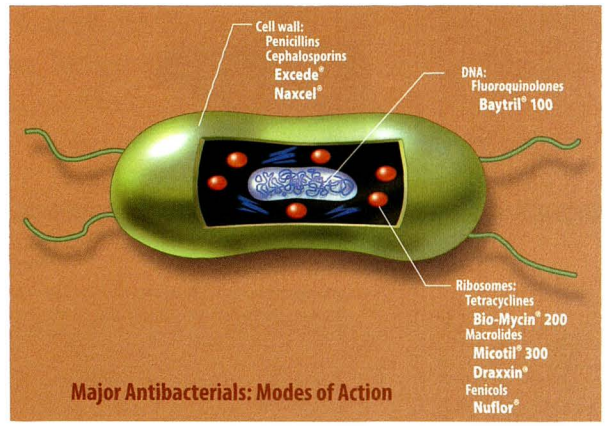
Baytril 100 is concentration-dependent: it achieves high drug levels at the site of infection to quickly kill bacteria.

Because BRD moves fast, you need an antibiotic that works fast. Some BRD treatments are time-dependent; they need to be at therapeutic levels at the site of the infection for a long period of time to be effective.

Baytril 100 is concentration-dependent, not time-dependent. It goes directly to the site of infection and starts killing BRD-causing bacteria in minutes, not days. Baytril 100 reaches therapeutic blood levels within 30 minutes, and therapeutic lung levels within 60 minutes.[1,2]



Baytril® 100 (enrofloxacin) is unique: it actually kills BRD-causing bacteria.



Many antibiotics are bacteriostatic. They do not kill bacteria immediately; they inhibit bacterial growth.

Baytril 100 is bactericidal. The unique mode of action of Baytril 100 allows it to penetrate the bacterial cell wall, disrupt the DNA and kill BRD-causing bacteria fast. And Baytril 100 kills bacteria in both the resting and growth phases of development.

Were food-safety questions addressed regarding the use of Baytril 100 in dairy replacement heifers?

Yes. The safety of Baytril 100 is well documented and food-safety questions were recently addressed through a Risk Assessment that estimated the potential risk to public health to be at or near zero.

Baytril 100 is an effective, lifesaving tool for the producer and veterinarian.

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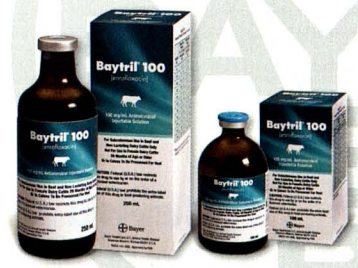
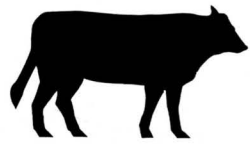


Baytril® 100

(enrofloxacin)



100 mg/mL Antimicrobial Injectable Solution

For Subcutaneous Use in Beef and Non-Lactating Dairy Cattle Only
Not For Use In Female Dairy Cattle 20 Months of Age or Older
Or In Calves To Be Processed For Veal

CAUTION:
Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.

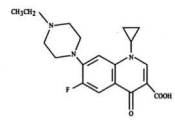
Federal (U.S.A.) law prohibits the extra-label use of this drug in food-producing animals.

PRODUCT DESCRIPTION:
Baytril® 100 is a sterile, ready-to-use injectable antimicrobial solution that contains enrofloxacin, a broad-spectrum fluoroquinolone antimicrobial agent.

Therapeutic treatment with Baytril® 100 may be administered as a single-dose or as a multiple-day therapy.

Each mL of Baytril® 100 contains 100 mg of enrofloxacin. Excipients are L-arginine base 200 mg, n-butyl alcohol 30 mg, benzyl alcohol (as a preservative) 20 mg and water for injection q.s.

CHEMICAL NOMENCLATURE AND STRUCTURE:
1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid.



INDICATION:
Baytril® 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni* (previously *Haemophilus somnus)* in beef and non-lactating dairy cattle.

DOSAGE AND ADMINISTRATION:
Baytril® 100 provides flexible dosages and durations of therapy.

Baytril® 100 may be administered as a single dose for one day or for multiple days of therapy. Selection of the appropriate dose and duration of therapy should be based on an assessment of the severity of disease, pathogen susceptibility and clinical response.

Single-Dose Therapy: Administer once, a subcutaneous dose of 7.5 - 12.5 mg/kg of body weight (3.4 - 5.7 mL/100 lb).

Multiple-Day Therapy: Administer daily, a subcutaneous dose of 2.5 - 5.0 mg/kg of body weight (1.1 - 2.3 mL/100 lb). Treatment should be repeated at 24-hour intervals for three days. Additional treatments may be given on days 4 and 5 to animals that have shown clinical improvement but not total recovery.

Administered dose volume should not exceed 20 mL per injection site.

Baytril® 100 Dose and Treatment Schedule for Cattle*

WEIGHT (lb)	Single-Dose Therapy 7.5 - 12.5 mg/kg Dose Volume (mL)	Multiple-Day Therapy 2.5 - 5.0 mg/kg Dose Volume (mL)
100	3.5 - 5.5	1.5 - 2.0
200	7.0 - 11.0	2.5 - 4.5
300	10.5 - 17.0	3.5 - 6.5
400	14.0 - 22.5	4.5 - 9.0
500	17.0 - 28.5	5.5 - 11.5
600	20.5 - 34.0	7.0 - 13.5
700	24.0 - 39.5	8.0 - 16.0
800	27.5 - 45.5	9.0 - 18.0
900	31.0 - 51.0	10.0 - 20.5
1000	34.0 - 57.0	11.0 - 23.0
1100	37.5 - 62.5	12.5 - 25.0

*Dose volumes have been rounded to the nearest 0.5 mL within the dose range.

RESIDUE WARNINGS:
Animals intended for human consumption must not be slaughtered within 28 days from the last treatment. Do not use in female dairy cattle 20 months of age or older. Use of enrofloxacin in this class of cattle may cause milk residues. A withdrawal period has not been established for this product in pre-ruminating calves. Do not use in calves to be processed for veal.

HUMAN WARNINGS:
For use in animals only. Keep out of the 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. For customer service or to obtain product information, including a Material Safety Data Sheet, call 1-800-633-3796. For medical emergencies or to report adverse reactions, call 1-800-422-9874.

PRECAUTIONS:
The effects of enrofloxacin on cattle reproductive performance, pregnancy and lactation have not been adequately determined.

Subcutaneous injection can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.

Baytril® 100 contains different excipients than other Baytril® products. The safety and efficacy of this formulation in species other than cattle have not been determined.

Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures. Quinolone-class drugs have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. See Animal Safety section for additional information.

ADVERSE REACTIONS:
No adverse reactions were observed during clinical trials.

MICROBIOLOGY:
Enrofloxacin is bactericidal and exerts its antibacterial effect by inhibiting bacterial DNA gyrase (a type II topoisomerase) thereby preventing DNA supercoiling and replication which leads to cell death.[1] Enrofloxacin is active against Gram-negative and Gram-positive bacteria.

EFFECTIVENESS:
A total of 845 calves with naturally-occurring BRD were treated with Baytril® 100 in eight field trials located in five cattle-feeding states. Response to treatment was compared to non-treated controls. Single-dose and multiple-day therapy regimens were evaluated. BRD and mortality were significantly reduced in enrofloxacin-treated calves. No adverse reactions were reported in treated animals.

TOXICOLOGY:
The oral LD50 for laboratory rats was greater than 5000 mg/kg of body weight. Ninety-day feeding studies in dogs and rats revealed no observable adverse effects at treatment rates of 3 and 40 mg/kg respectively. Chronic studies in rats and mice revealed no observable adverse effects at 5.3 and 323 mg/kg respectively. There was no evidence of carcinogenic effect in laboratory animal models. A two-generation rat reproduction study revealed no effect with 10 mg/kg treatments. No teratogenic effects were observed in rabbits at doses of 25 mg/kg or in rats at 50 mg/kg.

ANIMAL SAFETY:
Safety studies were conducted in feeder calves using single doses of 5, 15 and 25 mg/kg for 15 consecutive days and 50 mg/kg for 5 consecutive days. No clinical signs of toxicity were observed when a dose of 5 mg/kg was administered for 15 days. Clinical signs of depression, incoordination and muscle fasciculation were observed in calves when doses of 15 or 25 mg/kg were administered for 10 to 15 days. Clinical signs of depression, inappetance and incoordination were observed when a dose of 50 mg/kg was administered for 3 days. No drug-related abnormalities in clinical pathology parameters were identified. No articular cartilage lesions were observed after examination of stifle joints from animals administered 25 mg/kg for 15 days.

A safety study was conducted in 23-day-old calves using doses of 5, 15 and 25 mg/kg for 15 consecutive days. No clinical signs of toxicity or changes in clinical pathology parameters were observed. No articular cartilage lesions were observed in the stifle joints at any dose level at 2 days and 9 days following 15 days of drug administration.

An injection site study conducted in feeder calves demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue and underlying muscle. No painful responses to administration were observed.

STORAGE CONDITIONS: Protect from direct sunlight. Do not refrigerate, freeze or store at or above 40°C (104°F). Precipitation may occur due to cold temperature. To redissolve, warm and then shake the vial.

HOW SUPPLIED:

Baytril® 100:		
Code: 08711170-023699	100 mg/mL	100 mL Bottle
Code: 08711278-032199	100 mg/mL	250 mL Bottle

REFERENCES:

1. Hooper, D. C., Wolfson, J. S., *Quinolone Antimicrobial Agents*, 2nd ed, 59 - 75, 1993.

U.S. Patent No. 4,670,444

For customer service or to obtain product information, including a Material Safety Data Sheet, call 1-800-633-3796.
For medical emergencies or to report adverse reactions, call 1-800-422-9874.

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Baytril® 100

NADA 141-068, Approved by FDA

Bayer

Bayer HealthCare LLC
Animal Health Division
Shawnee Mission, Kansas 66201 U.S.A.

1. A study to compare the Plasma Pharmacokinetics of danofloxacin and enrofloxacin in Ruminating Cattle. Bayer Report 75646 (Bayer Study 151.603). © 2003 Bayer HealthCare LLC.
2. Concentration-dependent Killing Activity of Baytril® 100 (enrofloxacin). Bayer Study, BL04195. © 2004 Bayer HealthCare LLC.

BL08194

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