PEER REVIEWED

Serologic and other Diagnostic Evidence of *Neospora caninum* Presence in North Dakota Beef Herds

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Abstract

The objective of this study was to evaluate the presence of *Neospora caninum* antibodies in beef herds in North Dakota. A total of 212 cattle (97 calves and 115 cows) from seven cow-calf herds originating from four counties in North Dakota participated in the study. Blood was obtained from cattle by venipuncture and serum tested for *N. caninum* antibodies by indirect ELISA.

Additionally, data of *N. caninum* cattle cases were retrieved from NDSU Veterinary Diagnostic Laboratory (VDL) and analyzed. *N. caninum* cattle cases were characterized by location, time of diagnosis, age, diagnostic test and sample, and presenting syndrome. Serologic prevalence of *N. caninum* was 5.2% (5/97) and 5.2% (6/115) for calves and adult cattle, respectively.

Overall prevalence was 5.2% (11/212; 95% CI, 2.6%, 9.1%). Within-herd seroprevalence for cows and calves ranged from 0 to 13.3%, with a median prevalence of 3.3%. The 2004 VDL records indicated that 23 *N. caninum* cases were diagnosed in cattle, calves and fetuses of various breeds presenting with varying history. We concluded that *N. caninum* antibodies were present in the beef herds in ND, and may be as common in calves as in adult cattle. These findings underscore the need for further investigation of bovine neosporosis as a potential source of economic loss to the ND beef industry.

Résumé

L'objectif de cette étude était d'évaluer la présence d'anticorps contre *Neospora caninum* dans les troupeaux de boucherie du Dakota du Nord. Un total de 212 bovins (97 veaux and 115 vaches) provenant de sept troupeaux vaches-veaux localisés dans quatre comtés du Dakota du Nord ont été inclus dans l'étude. Des échantillons de sang ont été obtenus par ponction de la veine et le sérum à été testé pour la présence d'anticorps contre *N. caninum* par une méthode ELISA indirecte. Des données sur des cas bovins impliquant *N. caninum* ont de plus été recueillies au *NSDU Veterinary Diagnostic Laboratory* (VDL) et analysées. Les cas bovins impliquant *N. caninum* ont été classifiés selon leur localisation, le temps du diagnostic, l'âge, le test diagnostic et la présence du syndrome. La prévalence sérologique de *N. caninum* était de 5.2% (5/97) chez les veaux et de 5.2% (6/115) chez les bovins adultes. La prévalence sérologique dans son ensemble était de 5.2% (11/212) (I.C. 95% : 2.6%-9.1%). La prévalence sérologique à l'intérieur d'un troupeau pour les veaux et les bovins adultes variait de 0 à 13.3% avec une médiane de 3.3%. Les données du VDL en 2004 indiquaient la présence de 23 cas impliquant *N. caninum* chez des bovins, des veaux et des fœtus de races variées admis pour diverses raisons. Nous concluons que des anticorps contre *N. caninum* sont présents chez les bovins du Dakota du Nord et pourraient être aussi fréquents chez les veaux que chez les bovins adultes. Ces résultats soulignent le besoin de recherche plus poussée sur la néosporose bovine en tant que source potentielle de pertes de revenu pour l'industrie du bœuf au Dakota du Nord.

Introduction

Neospora caninum is a protozoal parasite of clinical importance in dogs[13,14] and cattle,[9,10] with the dog as the definitive host.[13,14] The parasite occasionally causes clinical infections in horses, goats, sheep and deer.[9] Animals including cattle, sheep, goats and deer act as intermediate hosts for this parasite. Transplacental transmission is considered the major route of infection in cattle.[1,9] Moreover, up to 95% of calves born congenitally infected with *N. caninum* remain clinically normal.[24] Horizontal infection of cattle resulting from the

ingestion of oocysts shed by dogs,[7,20] and possibly wildlife such as gray foxes and coyotes,[6] is also considered to be a significant route of *N. caninum* infection. A study[7] of patterns of *N. caninum* transmission in an epidemically infected beef herd estimated the efficiency of vertical transmission to be 85%, and several calves born to seronegative dams were seropositive at 6-13 months of age, indicating a 22% mean annual rate of horizontal transmission.

Neospora caninum is one of the most frequent infectious organisms causing abortion and congenital disease in cattle worldwide.[3,7,22,25] Reports indicate that abortion is the main consequence of fetal infection, but *in utero* fetal resorption, mummification or maceration, stillbirths, birth of calves with clinical signs, or clinically normal calves that are chronically infected may also result.[3,7,25] Risks of abortion, stillbirth, being culled for any reason or for reproductive failure have been reported to be greater in seropositive cows than in seronegative cows.[19,31] Bovine neosporosis has thus been identified as an important risk factor for reproductive losses in both beef and dairy cattle in many parts of the world,[7,15,16,18,19,22,31] including the United States (US).[3,4,11,21,23]

Published data on bovine neosporosis on the North American continent show the prevalence of the disease varies from place to place. Seroprevalence rates of 24.4 and 42.5% have been reported for California dairies,[2,3] while an infection rate of 80% in a single dairy herd with abortion problems was reported in South Carolina.[11] A survey of beef herds in the northwestern US indicated an overall prevalence of 23% and within-herd prevalence ranging from 2 to 67%.[23] A study[5] of the prevalence of *N. caninum* antibodies in Texas feeder cattle reported that 54 out of 92 (57.8%) consignments had at least one seropositive animal, while 131 out of 1,009 (13%) calves were seropositive. These investigators also reported that seropositivity to *N. caninum* antibodies was associated with a significant reduction in post-weaning weight gain and carcass weight. Studies done at different time points in the province of Alberta, Canada showed variable prevalence of *N. caninum* antibodies in beef herds, ranging from 5.9,[26] 6.5[27] to 9%.[28] Quantitative studies done in the US, New Zealand, the Netherlands and Germany indicated that 12 to 42% of the aborted fetuses from dairy cattle were infected with *N. caninum*,[9] while a study in Italy reported a prevalence of 11%.[18]

Although bovine neosporosis has been described worldwide, there is a lack of information concerning the prevalence of this infection in different cattle production systems.[19] Also, factors associated with the geographical distribution of seroprevalence, as well as the relative importance of vertical and horizontal transmission in the epidemiology of *N. caninum* in beef herds, are mostly unknown and require additional investigation.[23] A literature search for published data on the status of bovine neosporosis in North Dakota (ND) has shown very scanty information, yet the Veterinary Diagnostic Laboratory (VDL) at North Dakota State University (NDSU) continues to diagnose sporadic cases of the disease. The objective of this pilot study was to determine the presence of *N. caninum* in beef cattle herds in ND. The data generated could be used to design a more detailed epidemiological study to estimate the prevalence of *N. caninum* in beef herds in ND and determine factors that influence the occurrence of the disease under local conditions. Because endemic *N. caninum* infection has been shown to decrease return to fixed assets for cow-calf herds,[12] this information will be useful for both veterinarians and beef producers.

Materials and Methods

Animals and Blood Samples

Serum was collected from 212 cattle (97 calves and 115 cows) originating from seven cow-calf farms in four counties (Billings, Dunn, Mercer and Stark) in North Dakota. The cow-calf herds were enrolled in the ND Animal Identification project, and the owners were also willing to participate in this study. A random sample of at least 30 cattle (15 calves and 15 adult cows) was selected from each of the seven herds that participated in the study, except where less than 30 animals in each category were available. In these cases, all cattle were sampled. One herd had only calves and two herds had only adult cows, and so 30 animals of one category were sampled from each of these herds. Blood was collected by venipuncture, brought to the laboratory at NDSU, left to clot at room temperature (RT) overnight and the separated serum frozen at -20°C (-4°F) until testing approximately four weeks later.

Neospora *Enzyme Linked Immunosorbent Assay (ELISA)*

The 212 serum samples were tested by indirect ELISA (iELISA) for *N. caninum* antibodies, as described in the IDEXX *Neospora*-ELISA kit protocol.[17] Test sera was diluted 1:100 in phosphate buffered saline-Tween-20 1x, pH 7.4 (PBS-T), and positive and negative control samples were incubated at room temperature (RT) for 30 minutes in *N. caninum* antigen-precoated, 96-well microtiter plates. After four washes with PBS-T, 100 µl of PBS-T diluted anti-bovine horseradish peroxidase conjugate (HRPO) was added per well and incubated at RT for 30 minutes. Following the incubation step, the plate was washed as before and incubated further at RT with 100 µl/well of 3,3', 5,5'-tetramethyl benzidine substrate (TMB). The reaction was stopped with 100 µl of stop solution and the plates read at 650 nm within 30 minutes. The data were analyzed using soft-

ware supplied with the ELISA reader that calculates normalized optic density (OD) readings as follows: normalized OD = (raw OD of sample – raw OD of negative control) / (raw OD of positive control – raw OD of negative control).

Diagnostic Data from NDSU-VDL

Data on bovine cases diagnosed as positive for *Neospora caninum* by NDSU-VDL in 2004 were accessed. Additionally, information regarding clinical history, age, breed and county of origin of each case was obtained.

Data Analysis

Geographic Information Systems Arc Info 8 software[a] was used to show the spatial distribution by county of bovine cases of *N. caninum* detected in our study, as well as those reported by VDL in 2004 in ND. Descriptive statistics of animals that tested positive to *N. caninum* were computed in Microsoft Excel version 5. *N. caninum* cases reported at the VDL in 2004 were characterized by time of diagnosis, age, diagnostic test and sample used, and history of the case.

Results

N. caninum *Results from Sample Population*

Results from the *Neospora* ELISA indicated an apparent prevalence of *N. caninum* antibodies of 5.2% (5/97) in calves and 5.2% (6/115) in adult cattle, with an overall prevalence of 5.2% (11/212; 95% CI, 2.6 %, 9.1%; Table 1) in the sample population. Within-herd seroprevalence ranged from 0 to 13.3%, with a median of 3.3%. All animals in three of the seven herds tested negative to *N. caninum* antibodies. All four counties (Mercer, Stark, Billings, Dunn) involved in the study had at least one animal test positive to *N. caninum* antibodies, with the total number of animals positive per county ranging from one to four (Figure 1).

Table 1. Frequency distribution of cattle that tested positive to *Neospora caninum* by county and age category - North Dakota, 2004.

		Neospora caninum	
County	Age group	Positive samples	Percent positive
Mercer	calves	0 of 15	0.0
	adults	1 of 15	6.7
Stark	calves	0 of 41	0.0
	adults	4 of 51	7.8
Billings	calves	3 of 26	11.5
	adults	1 of 4	25.0
Dunn	calves	2 of 15	13.3
	adults	0 of 45	0.0
Totals	calves	5 of 97	5.2
	adults	6 of 115	5.2
Overall	cattle	11/212	5.2

N. caninum *Cases Reported by VDL*

In 2004, a total of 23 bovine *N. caninum* infections were diagnosed in cattle originating from eight out of 53 counties in North Dakota (Figure 1). Diagnosis of the 23 positive *Neospora* cases was based on laboratory tests as follows: 21 were diagnosed by ELISA testing of adult cattle serum; one by ELISA testing on fetal thoracic fluid; and one case on the basis of positive immunohistochemical (IHC) staining for *N. caninum* antigens in fetal brain tissue. VDL records indicated that several beef and dairy breeds were affected by *N. caninum,* including Simmental, Angus, Holstein, mixed breed, Red Angus and Gelbvieh. Of the 10 cases where animal age was provided, eight were adult cattle while the other two were aborted fetuses. Eleven of 23 (48%) cases of *N. caninum* diagnosed at VDL had a history of abortion; the rest were submitted to VDL due to poor herd conception rates (2/23, 8.7%) or for voluntary Johne's disease herd evaluation (10/23, 43.5%), with *Neospora* testing being requested as an additional screening test. In 2004, *N. caninum* cases were reported in four of 12 months (April, July, November and December), with the majority occurring in November (11/23, 48%) and December (7/23, 30%), respectively.

Discussion

Antibodies to *N. caninum* were found in at least one animal from each county where we sampled, an indication of potential widespread exposure of the ND beef cattle to *N. caninum.* It has been reported that exposure to *N. caninum* antigen is common in beef cow-calf herds in the northwestern US.[23] A previous study[23] of *Neospora* seroprevalence in five northwestern states reported widespread presence of *Neospora* infection in many northwestern US beef cattle herds, as all 55 study herds for which blood samples were returned showed evidence of exposure to *Neospora*. Overall seroprevalence was 24% and within-herd seroprevalence ranged from 3 to 67%, with a median of 19%.[23] Another study[21] of dairy and beef herds from 20 US states and the territory of Puerto Rico, using commercially available ELISA to test for antibodies to *N. caninum*, showed that 16% of cows tested positive. At least one seropositive animal was detected in 90% of the herds tested, and prevalence of cows seropositive to *N. caninum* varied from 2 to 65% among herds.[21] A study[5] of Texas beef calves reported that 99 of 760 (13%; 95% CI, 9.4%, 17.7%) calves were

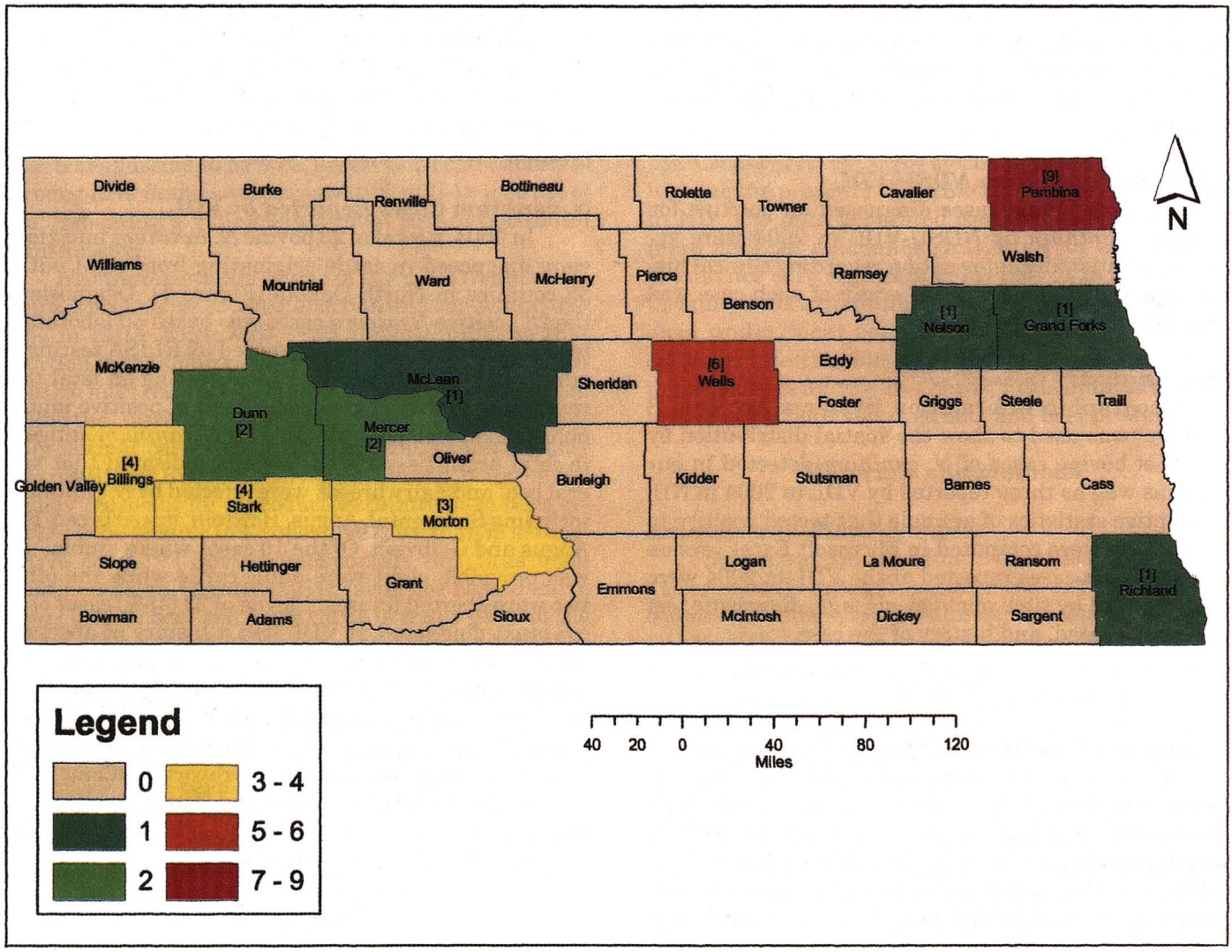

Figure 1. Distribution of *Neospora caninum* cases by county as reported from samples we tested and by the Veterinary Diagnostic Laboratory (VDL). North Dakota, 2004

positive to *N. caninum* and 59% of the ranches consigned at least one positive calf.

Other studies conducted outside the US have reported widespread occurrence of *N. caninum* in both beef and dairy production systems.[7,19,29-31] One study[31] found the seroprevalence at cross-sectional samplings ranged from 16 to 27% in eight Canadian beef herds and, unlike this study, all herds had seropositive animals. In yet another Canadian study,[29] blood samples were collected from 1806 pregnancy-tested cows from 174 herds at a northern Alberta auction mart in the fall of 1998, and 162 (9.0%) of these samples were positive for antibodies to *N. caninum*. A study[19] that investigated the seroprevalence of *N. caninum* infection in beef and dairy cattle production in Spain detected specific antibodies to this organism in 55.1% (119/216) of beef and 83.2% (119/143) of dairy herds. Individual prevalence values were 17.9% (306/1712) for beef and 35.9% (402/1121) for dairy animals.[19]

Overall seroprevalence in our study (5.2%) and within-herd prevalence (range 0 to 13.3%, median 3.3%) was lower than reported in most studies. It is possible that our comparatively smaller sample size limited our ability to estimate the true prevalence of *Neospora* exposure in the herds we sampled. At the same time, because of the chronicity and widespread nature of bovine neosporosis, it is possible that the randomized sample size of 212 provided adequate prevalence estimates for the herds sampled. Moreover, relative sensitivity of one-time serological sampling for *N. caninum*, compared to three consecutive samplings, was reported as 94.7%, while relative specificity was 95.6%.[8] Predictive values positive and negative of one-time sampling were 92.4 and 97%, respectively, and agreement between one-time

sampling and three consecutive samplings, kappa, was 0.90.[8] Additionally, some of the studies[7,30] sampled cattle with reproductive disease, while our study did not. A study[16] that evaluated the seroprevalence of *Neospora* in cows without reproductive disease reported a prevalence of 4.7% (19/400) for beef cattle, similar to the 5.2% prevalence reported in this study.

Given the relative sensitivity and specificity of one-time serological sampling reported, the potential for misclassification of animals and/or herds in this study could not be ruled out. Animals or herds classified falsely as negative could perpetuate infection and possibly cause abortions in adult cattle and poor post-weaning weight gain in calves. Also, false positive animals or herds could cost producers because of possible unnecessary culling.

Our study found prevalence of antibodies to *N. caninum* in adult cattle similar to that in calves. This finding was not a total surprise since transplacental transmission is considered the major route of infection for bovine neosporosis, and most seropositive cows are expected to produce seropositive calves.[27,30] Evidence of cow-to-fetus transmission of the organism has been detected in more than 82% of seropositive cows.[30] However, the role of vertical transmission in the epidemiology of *N. caninum* was not examined in this study because dam-daughter connections were not maintained in most herds.

There was an apparent seasonal variation in the number of *N. caninum* cases diagnosed at the VDL, with the majority of cases occurring in November and December of 2004. A study[25] that evaluated the secular and seasonal trends of *Neospora* abortion in dairy cows in California reported that more abortions were likely to occur in winter than in summer and early fall. Moreover, unpublished data at the NDSU-VDL suggest that most bovine abortions in the state occur during the winter months. It is possible that this observation may be related to stress caused by suboptimal winter temperatures, or that breeding of cattle is usually done so that most animals are in the second trimester of pregnancy in the months of November/December. In addition, abortions are more noticeable in late gestation, and producers are in closer contact with their animals in the late fall and winter months following the grazing season. With very few published reports, however, the validity of this observation warrants further investigation.

VDL records, which are similar to other reports, indicate both beef and dairy cattle in North Dakota are at risk for infection with this parasite.[3,21,25] VDL data appeared to suggest that *Neospora* infection might be associated with bovine abortion in North Dakota.

Conclusions

We concluded that *N. caninum* antibodies were present in the beef herds sampled, an indication that *N. caninum* infection may be present in beef cattle herds in ND. Based on these findings, bovine neosporosis should be investigated as a potential source of economic loss to the North Dakota beef industry.

Acknowledgement

Funded by a grant from USDA-APHIS. The authors wish to thank the staff of Dickinson Research Extension Center for their technical assistance, and North Dakota beef producers for participating in the study.

Endnotes

[a] Geographic Information Systems, ArcInfo 8, ESRI, Redlands, Calif.

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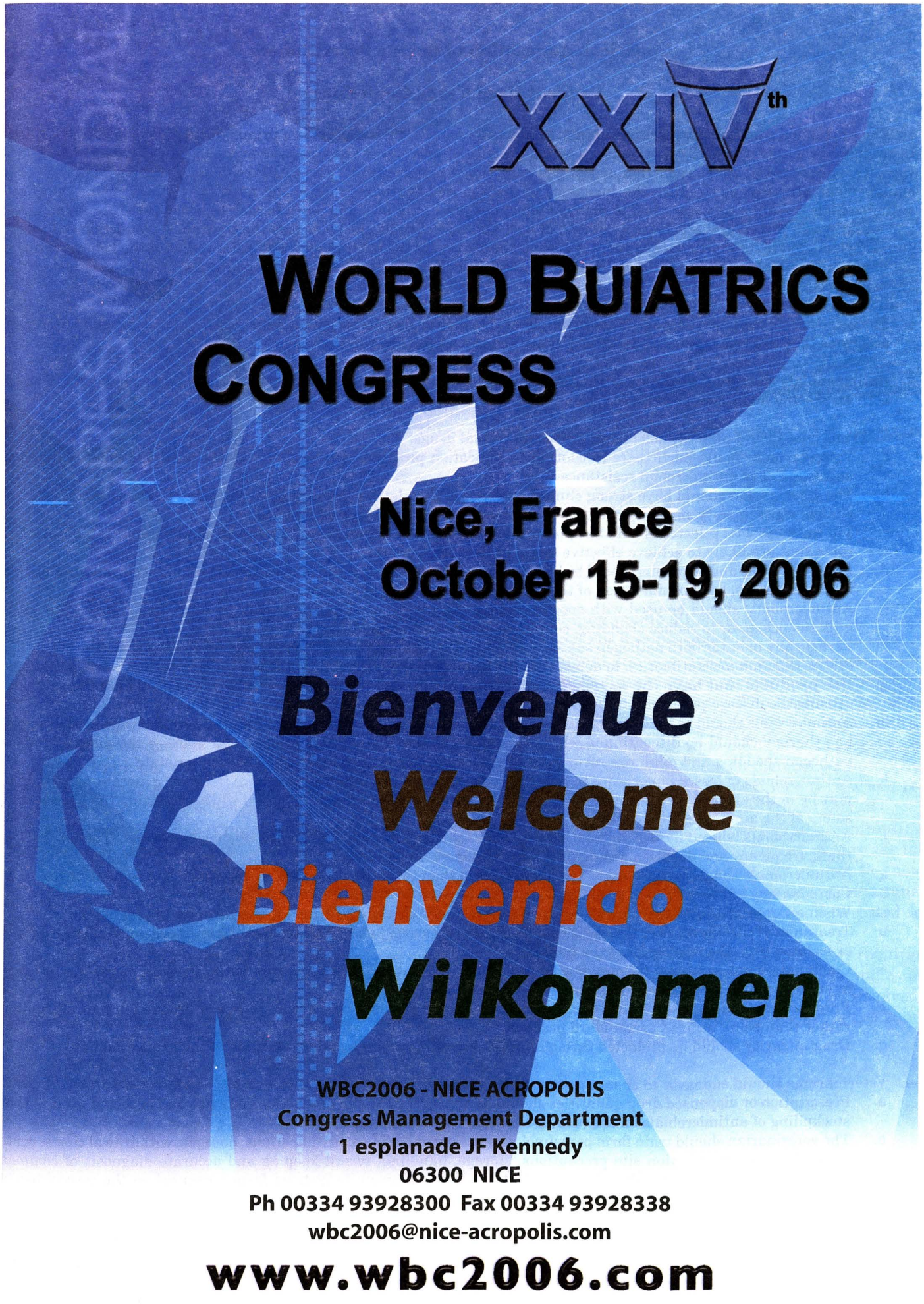

American Association of Bovine Practitioners

Prudent Drug Usage Guidelines

The production of safe and wholesome animal products for human consumption is a primary goal of members of the AABP. In reaching that goal, the AABP is committed to the practice of preventive immune system management through the use of vaccines, parasiticides, stress reduction and proper nutritional management. The AABP recognizes that proper and timely management practices can reduce the incidence of disease and therefore reduce the need for antimicrobials; however, antimicrobials remain a necessary tool to manage infectious disease in beef and dairy herds. In order to reduce animal pain and suffering, to protect the economic livelihood of beef and dairy producers, to ensure the continued production of foods of animal origin, and to minimize the shedding of zoonotic bacteria into the environment and potentially the food chain, prudent use of antimicrobials is encouraged. Following are general guidelines for the prudent therapeutic use of antimicrobials in beef and dairy cattle.

1. The veterinarian's primary responsibility to the client is to help design management, immunization, housing and nutritional programs that will reduce the incidence of disease and the need for antimicrobials.

2. Antimicrobials should be used only within the confines of a valid veterinarian-client-patient relationship; this includes both dispensing and issuance of prescriptions.

3. Veterinarians should properly select and use antimicrobial drugs.
   a. Veterinarians should participate in continuing education programs that include therapeutics and emerging and/or development of antimicrobial resistance.
   b. The veterinarian should have strong clinical evidence of the identity of the pathogen causing the disease, based upon clinical signs, history, necropsy examination, laboratory data and past experience.
   c. The antimicrobial selected should be appropriate for the target organism and should be administered at a dosage and route that are likely to achieve effective levels in the target organ.
   d. Product choices and regimens should be based on available laboratory and package insert information, additional data in the literature, and consideration of the pharmacokinetics and pharmacodynamics of the drug.
   e. Antimicrobials should be used with specific clinical outcome(s) in mind, such as fever reduction, return of mastitic milk to normal, or to reduce shedding, contagion and recurrence of disease.
   f. Periodically monitor herd pathogen susceptibility and therapeutic response, especially for routine therapy such as dry cow intramammary antibiotics, to detect changes in microbial susceptibility and to evaluate antimicrobial selections.
   g. Use products that have the narrowest spectrum of activity and known efficacy *in vivo* against the pathogen causing the disease problem.
   h. Antimicrobials should be used at a dosage appropriate for the condition treated for as short a period of time as reasonable, i.e., therapy should be discontinued when it is apparent that the immune system can manage the disease, reduce pathogen shedding and minimize recurrence of clinical disease or development of the carrier state.
   i. Antimicrobials of lesser importance in human medicine should be used in preference to newer generation drugs that may be in the same class as drugs currently used in humans if this can be achieved while protecting the health and safety of the animals.
   j. Antimicrobials labeled for use for treating the condition diagnosed should be used whenever possible. The label, dose, route, frequency and duration should be followed whenever possible.
   k. Antimicrobials should be used extra-label only within the provisions contained within AMDUCA regulations.
   l. Compounding of antimicrobial formulations should be avoided.
   m. When appropriate, local therapy is preferred over systemic therapy.
   n. Treatment of chronic cases or those with a poor chance of recovery should be avoided. Chronic cases should be removed or isolated from the remainder of the herd.
   o. Combination antimicrobial therapy should be discouraged unless there is information to show an increase in efficacy or suppression of resistance development for the target organism.
   p. Prophylactic or metaphylactic use of antimicrobials should be based on a group, source or production unit evaluation rather than being utilized as standard practice.
   q. Drug integrity should be protected through proper handling, storage and observation of the expiration date.

4. Veterinarians should endeavor to ensure proper on-farm drug use.
   a. Prescription or dispensed drug quantities should be appropriate to the production-unit size and expected need so that stockpiling of antimicrobials on the farm is avoided.
   b. The veterinarian should train farm personnel who use antimicrobials on indications, dosages, withdrawal times, route of administration, injection site precautions, storage, handling, record keeping and accurate diagnosis of common diseases. The veterinarian should ensure that labels are accurate to instruct farm personnel on the correct use of antimicrobials.
   c. Veterinarians are encouraged to provide written guidelines to clients whenever possible to describe conditions and instructions for antimicrobial use on the farm or unit.

Presented by the Bacterial Resistance and Prudent Therapeutic Antimicrobial Use Committee. Board approved March 1999.