Parasite Resistance in US Cattle

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Abstract

Parasite resistance to the macrocyclic lactones (ivermectin, doramectin, eprinomectin, and moxidectin) is receiving considerable attention in the US cattle industry at a time when the economics of parasitism constitute one of the most important factors involved in beef production. Knowing whether a dewormer is effective is extremely important to an operation. If parasites become resistant to a particular product or product formulation, a serious problem can develop unknowingly unless producers have an easy way to determine product efficacy. The fecal egg count reduction test (FECRT) is a simple test recommended by the American Association of Veterinary Parasitologists (AAVP) as the best way for the practitioner to help producers verify that the dewormer(s) they are using is effective. The FECRT involves conducting a fecal check at the time of treatment and again 14 days following treatment. In the fall of 2007 continuing through the summer of 2008, free lab support was offered to bovine practitioners throughout the US to conduct FECRTs with their clients. This was done according to a standard protocol involving a minimum of 20 samples per treatment group at each collection time. The results are being recorded in a national data base supported by Intervet/Schering-Plough Animal Health and the University of Nevada-Reno. Over 58 veterinary clinics in 19 states have already participated in this program, with over 119 separate tests involving 4,765 samples using a wide range of products and formulations. These data confirm that macrocyclic lactone resistance is widespread and that continued vigilance is required by the veterinary profession, since the problem now appears to be at a critical stage with millions of dollars in production losses at stake.

Résumé

La résistance des parasites aux lactones macrocycliques (ivermectine, doramectine, éprinomectine et moxidectine) attire beaucoup l'attention de l'industrie du bétail aux États-Unis à un moment où les retombées économiques des parasites constituent l'un des principaux facteurs touchant la production bovine. Il est extrêmement important qu'une opération connaisse l'efficacité des vermicides. Si les parasites développent une résistance à un produit particulier ou à une formulation particulière du produit, un sérieux problème peut s'ensuivre à l'insu des producteurs sauf si ces derniers ont un moyen simple de déterminer l'efficacité du produit. Le test de la réduction du nombre d'œufs fécaux est un simple test recommandé par l'American Association of Veterinary Parasitologists (AAVP) qui permet aux praticiens d'aider les producteurs à déterminer le plus facilement si les vermicides qu'ils utilisent sont encore effectifs. Ce test fécal implique une vérification fécale au moment du traitement et ensuite 14 jours après traitement. Entre l'automne 2007 et l'été 2008, l'accès gratuit au laboratoire a été offert aux praticiens bovins à la grandeur des États-Unis afin de leur permettre de faire le test fécal pour leurs clients. Un protocole standardisé a été adopté impliquant un minimum de 20 échantillons par groupe de traitement aux deux moments d'échantillonnage. Les résultats sont consignés dans une banque de données nationale sous la tutelle d'Intervet/Schering-Plough Animal Health et de l'université de Nevada-Reno. Plus de 58 cliniques vétérinaires dans 19 états participent déjà au programme. Il y a plus de 119 tests distincts impliquant 4765 échantillons avec une panoplie de produits et de formulations. Ces données confirment que la résistance aux lactones macrocycliques est étendue et il faut donc une vigilance soutenue de la part du corps professionnel vétérinaire car le problème semble maintenant au point critique et il y a des pertes de millions de dollars de production en jeu.

Introduction

Deworming beef and dairy cattle in the US has evolved over the past 25 years to become a standard recommended practice on many progressive operations, with emphasis on the economic benefits of deworming. Each year, more producers are preventively deworming their cattle at strategic times of the year to prevent economic losses caused by parasitism, rather than waiting to deworm cattle until after these animals are harboring heavy burdens and significant damage to the animals has already occurred. Most producers are concerned about deworming at the optimal time to achieve maximum benefit. These producers appreciate having highly efficacious formulations that are safe and easy to apply, and trust that the efficacy claim approved for the dewormer used is reliable.

The economic importance of parasitism is changing as animal production becomes more efficient due to continued improvements in genetic, nutrition, implant

technologies, and disease control measures. A recent study from Iowa State University identified parasite control as the single most important economic factor in producing beef efficiently. This study identified parasites as a major detriment to efficient production and that parasites are responsible for adding as much as $190 per animal to the cost of raising beef cattle.[25] The economics of parasitism calculated for this analysis came from the effects of parasitism upon reproductive efficiency, rate of gain, feed efficiency, carcass quality, milk production and the immune system through reduced mortality and morbidity.[31,32]

It is apparent that as animals become more efficient, it takes fewer parasites to cause economic loss than in less efficient animals. Studies at the University of Wisconsin demonstrated that cows in early lactation had greater production loss due to parasite exposure than cows exposed to parasites later in lactation when production stresses were much less.[5] A second study at the University of Wisconsin showed that improvement in milk production due to deworming was greatest in the best managed herds.[4] It takes fewer parasites, therefore, to cause economic loss in a dairy cow milking 30,000 lb (13,636 kg) of milk per lactation than one milking 15,000 lb (6,818 kg) per lactation or in a feedlot animal gaining 4.4 lb (2 kg) per day versus an animal gaining 2.2 lb (1 kg) per day. The more efficient an animal is, the greater impact parasites can have on maintaining this efficiency. When parasites are missed by an inefficient dewormer or because of anthelmintic resistance, unless detected quickly, these parasites can be very damaging to an operation not only through production losses, but also by the continued contamination of the animal's environment ensuring future infections.

Based on research conducted on the benefits of strategically timed deworming, considerable efforts have been made to teach veterinarians, nutritionists, pharmaceutical representatives, feed company representatives and producers about these benefits.[6,8,11,22,31,32,33] A number of companies have created FDA approved formulations that facilitate the ease of deworming for the producer. These formulations include many non-handling forms such as medicated blocks, medicated free-choice minerals, medicated range cube or cake supplements, medicated complete feeds and top-dressed feed formulations, liquid supplements as well as topically applied pour-ons.[3,4,12,21,34]

The goal of strategically timed anthelmintic application is to prevent economic loss and reduce environmental parasite contamination by eliminating worm-egg shedding for a period of time at least equal to the life cycle of the parasites removed.[1,6,20] This strategy entails more than simply applying a dewormer. The timing of the deworming is very important, and things to be considered include the season of the year, type of grazing programs practiced and the overall management goals of the operation. The success or failure of these strategically timed programs depend upon a number of factors, one of the most important being the ability of the anthelmintic to stop parasite eggs being shed back on the pastures, especially during the early part of the grazing season. If the anthelmintic fails to stop worm-egg shedding and cattle continue to shed worm eggs back on the pasture following treatment, the potential for pasture cleanup is greatly reduced or, in many cases, eliminated.[1,18,19,28,29]

The failure of the endectocide pour-ons to eliminate worm egg shedding was identified soon after the endectocide pour-ons were first introduced on the U.S. market.[8,16,23,34] This continual shedding predisposed the surviving parasites and their progeny to develop resistance to the macrocyclic lactone (ML) compounds used in the pour-on formulations. Since parasite survival and continual egg shedding is occurring while these chemical compounds are still active in the animals and their feces, both the worms themselves and the eggs being shed on the pasture are exposed to the chemical residue of the compounds in the feces. This reduced efficacy and continual product exposure by the parasites over time creates the potential for parasite resistance to develop to these compounds.[13,14,27] This problem is compounded by the "persistent efficacy" feature by these pour-on products. Based on FDA approvals, these products exhibit persistent residues in the animals ranging from 14 to 42 days following treatment depending upon the product involved. The persistent residues indicate prolonged exposure of the surviving parasites in the gastrointestinal tract and parasite offspring (larvae) surviving in the manure to the ML compounds, thereby greatly increasing the chance for development of parasite resistance to these compounds.[2] Recent data, in fact, indicate that parasite resistance is now a real threat in operations where ML pour-ons have been used for several years.[10,14,31]

The reason for the reduced efficacy with ML pour-ons has been identified as the lack of consistent and adequate level of absorption by the endectocide pour-ons into the bloodstream, when compared to injectable formulations of the same products.[15] Blood level determinations following treatment with doramectin in an injectable formulation demonstrated 90% absorbed while the pour-on formulation was only 15% absorbed. Absorption data is given as follows: 200 mg/kg injectable ML will deliver a maximum plasma concentration with a mean of 32ng/ml, while a 500 mg/kg pour-on ML will deliver a maximum plasma concentration with a mean of 12ng/ml. This reduced blood level (12ng/ml versus 32ng/ml) indicates that many animals may not be receiving a therapeutic dose following treatment with the ML pour-on formulations and the parasites

and their offspring are predisposed to possible parasite resistance. Also, the adult parasites and newly developing adults that survive pour-on treatment continue to produce eggs that are shed back into the environment of the animals, making these ML pour-ons unsuitable for use in a strategic deworming program.

The history of the detection of anthelmintic resistance in cattle began as early as 1997 when a FECRT conducted in New Zealand showed that the ML pour-ons (moxidectin and ivermectin) failed to control parasites as well as an ML injectable formulation (doramectin).[17] Then in 1999, a FECRT conducted in Louisiana showed weekly samples taken for eight weeks following treatment with ivermectin pour-on and doramectin pour-on ranged from 50 to 79% efficacy for doramectin and 43% to 85% for ivermectin.[37] This study demonstrated that parasite resistance was already present in Louisiana. The first field study where parasite resistance was confirmed with worm counts at necropsy in a critical efficacy study was conducted in Wisconsin.[16,32] In this study, the efficacy of doramectin, moxidectin, eprinomectin and Ivomec® Plus (Merial) was tested. Comparing worm counts to non-medicated control cattle, the efficacy of moxidectin was 88.0%, doramectin was 64.1%, eprinomectin was 73.1% and Ivomec® Plus was 0%. All four compounds were identified as resistant, with efficacies far below the desired efficacy of 90% or greater.[32]

Eprinomectin and moxidectin were further investigated using the FECRT protocol in two separate commercial beef herds owned by the University of Illinois at the Dixon Spring Agricultural Station in Simpson, Illinois to investigate whether the repeated use of eprinomectin or moxidectin would lead to parasite resistance.[16] In the first phase of the first trial, 30 animals in each herd received eprinomectin pour-on according to label directions (0.5mg/kg BW). In the second phase, treated animals from the first trials were ranked based on post-treatment worm egg counts, blocked and randomly assigned to one to two treatment groups. Fifteen animals from each herd received eprinomectin pour-on (0.5mg/kg BW), while the remaining 15 animals from each herd received fenbendazole oral paste (5mg/kg BW). In the first phase of the second trial, 30 animals in each herd received moxidectin pour-on according to label directions (0.5mg/kg BW). In the second phase, treated animals from the first trials were ranked based on post-treatment worm-egg counts, blocked and randomly assigned to one to two treatment groups. Fifteen animals from each herd received moxidectin pour-on (0.5mg/kg BW), while the remaining 15 animals from each herd received fenbendazole oral paste (5mg/kg BW).

Results demonstrated that an efficacy value of 84.8% was achieved for eprinomectin in Phase 1 of the first trial and an efficacy value of 5.5% in the second phase of the first trial. Results demonstrated that an efficacy value of 74.7% was achieved for moxidectin in Phase 1 of the second trial and an efficacy value of 0% in the second phase of the second trial. Fenbendazole maintained an efficacy value of greater than 95% in both trials. The fecal worm-egg count results from this study revealed that the parasites which survived the first ML treatment were refractory to a second ML treatment, indicating that the ML pour-ons selected for resistant parasites during the first exposure were then resistant to further treatment by any ML compound.

Materials and Methods

The fecal worm egg reduction test (FECRT) is now recommended as a field test to determine whether treatment is successful and that a FECRT with efficacy less than 90% indicates that anthelmintic resistance is present.[13,35,37] In the fall of 2007 and continuing through the end of the summer of 2008, a nationwide survey was set-up to determine the scope and scale of ML resistance. FECRTs were offered free to veterinary clinics all across the US by Intervet/Schering-Plough Animal Health as a valuable tool for practitioners to test whether a particular product of choice was working for their clients. A standard protocol was provided for each participating clinic to use.

Each participating clinic would identify a cooperating producer with a minimum of 20 parasitized animals between six months and two year olds to conduct each test. All sampling was done under the supervision of the participating clinic. Each participating veterinary clinic was offered two trials per clinic conducted free, plus each clinic received additional compensation for their time involved in setting up and conducting the tests. Samples were collected at the time of treatment and again 14-days later. These samples were kept cool and sent with ice packs to one of three separate parasitology labs for analysis using the Modified Wisconsin Sugar Flotation Method.[11] All samples were blinded to treatment, and pre-treatment and post-treatment samples from the same location were sent to the same lab.

Results

Fifty-eight veterinary clinics located in 19 states have participated in the survey on parasite resistance, conducting 119 FECRTs involving 4,765 samples. The efficacy of the injectable ML formulations was tested in 26 tests showing the efficacy of Ivomec® (Merial) at 76.2%, Ivomec® Plus (Merial) at 42.6%, Dectomax® (Pfizer, Inc.) at 89.9%, Cydectin® (Ft Dodge Animal Health) at 98.1% and ivermectin (generic) at 50.0%. The overall efficacy of the ML injectable formulations was 72.5% (Table 1). The efficacy of the ML pour-on formulations was tested in 60 tests showing the efficacy of Ivomec®

Table 1. Efficacy of macrocyclic lactone injectable formulations from FECRTs* conducted by veterinary practitioners and submitted to Intervet's national database.

Product	Number of trials	Number of samples	Egg counts/3g** Pre-Rx	Post-Rx	Percent efficacy (%)
Injections:					
Ivomec® Inj.	6	162	55.5	13.2	76.2%
Ivomec® Plus	6	257	120.4	69.1	42.6%
Dectomax® Inj.	11	362	43.6	4.4	89.9%
Cydectin Inj.	2	64	246.1	4.7	98.1%
Ivermectin Inj.	1	40	33.0	16.5	50.0%
Inj. Summary:	26	884	79.2	21.8	72.5%

*Fecal egg count reduction tests.
** All samples taken at treatment and again two weeks post-treatment.

Table 2. Efficacy of macrocyclic lactone pour-ons from FECRTs* conducted by veterinary practitioners and submitted to Intervet's national database.

Product	Number of trials	Number of samples	Egg counts/3g** Pre-Rx	Post-Rx	Percent efficacy (%)
Pour-ons:					
Ivomec® PO	8	366	45.8	12.7	72.3%
Ivermectin PO	35	1,437	53.6	21.6	59.7%
Dectomax® PO	8	318	89.2	18.8	78.9%
Cydectin® PO	9	365	45.1	14.8	67.2%
Pour-On summary	60	2,486	56.0	19.0	66.1%

* Fecal egg count reduction tests.
**All samples taken at treatment and again two weeks post-treatment.

at 72.3%, ivermectin (generic) at 59.7%, Dectomax® at 78.9% and Cydectin® at 67.2%. The overall efficacy of the ML pour-ons was 66.1% (Table 2). The efficacy of Safe-Guard®/Panacur® was tested in 24 tests with 1,016 samples with a mean pre-treatment egg count of 67eggs/3gm and a mean post-treatment egg count of 0.4 egg/3gm for an overall efficacy of 99.4% (Table 3). In nine trials, a combination treatment was given, with either Safe-Guard® or Panacur® given at the same time as a ML injectable or ML pour-on formulation with either Ivomec®, Dectomax®, or Cydectin®. These tests involved 261 samples, with a mean pre-treatment count of 152.1 eggs/3gm and a mean post-treatment count of 0.1 egg/3gm for an overall efficacy of 99.9% mean (Table 4).

Discussion

The World Association for the Advancement of Veterinary Parasitology (WAAVP) has defined anthelmintic resistance to any product as efficacy values of below 90%.[15,38] Positive worm-egg counts two weeks following treatment indicate incomplete kill, however, egg counts don't identify the size of the residue population of parasites which remain in an animal after treatment. An extensive feedlot production study involving over 700 yearling cattle showed that a mean fecal worm-egg count of 9.0 epg decreased gain by 4.2%, while a high worm burden with a mean fecal worm egg count of 47.0 epg decreased gain by 13.3%.[33] The overall summary of all ML injectable formulations in 26 tests involving 884 cattle was a mean egg count of 21.8 eggs/3gm and 19.0 eggs/3gm for the ML pour-ons in 60 tests conducted with 2,486 cattle. Since these tests were conducted across 19 states, it is evident that ML resistance is now widespread and the cost of ML resistance to US cattle producers may be in the millions of dollars.

The dilemma which occurs for the US cattle producer is that for many, ML formulations are an important part of their arsenal of products used to control

Table 3. Efficacy of various Safe-Guard®/Panacur® formulations from FECRTs* conducted by veterinary practitioners and submitted to Intervet's national database.

Product	Number of trials	Number of samples	Egg counts/3g** Pre-Rx	Egg counts/3g** Post-Rx	Percent efficacy (%)
Panacur® drench	7	267	65.9	0.2	99.7%
SG drench	10	335	103.3	0.5	99.5%
Summary drench	17	602	87.9	0.3	99.7%
SG feed	5	314	41.7	0.3	99.4%
SG mineral	1	60	4.0	0.5	88.9%
SG paste	1	40	33.9	0	100.0%
Safe-Guard®/Panacur® Overall summary**	24	1,016	67.0	0.4	99.4%

* Fecal egg count reduction tests.
**All samples taken at treatment and again two weeks post-treatment.

Table 4. Efficacy of Safe-Guard®/Panacur® in combination with various macrocyclic lactone formulations from FECRTs* conducted by veterinary practitioners and submitted to Intervet's national database.

Combination product	Number of trials	Number of samples	Egg counts/3g** Pre-Rx	Egg counts/3g** Post-Rx	Percent efficacy (%)
Safe-Guard/Panacur Drench plus:					
Ivomec® Inj.	3	59	88.2	0	100.0%
Ivomec® Plus	1	40	30.7	0	100.0%
Ivermectin PO	3	118	30.8	0.1	99.9%
Dectomax® Inj.	1	20	389.4	0	100.0%
Cydectin® Inj.	1	24	583.0	0.2	99.9%
Summary	9	261	152.1	0.1	99.9%

* Fecal egg count reduction tests.
**All samples taken at time of treatment and again two weeks post-treatment.

external parasite (lice, mites, grubs and flies). In nine tests in 261 cattle, where fenbendazole was given at the same time as a ML injectable or ML pour-on formulation, the FECRT indicated a mean efficacy of 99.9% across all nine tests (Table 4). From these tests, it appears that whenever a ML formulation is used for external parasite control, it should be used simultaneously with a non-ML internal parasiticide to prevent losses due to internal parasitisms and to prevent the further transfer of ML resistance parasites to other cattle.

References

1. Armour J, Bairden K, Duncan JL, Jones RM., Bliss DH: Studies on the control of bovine ostertagiasis using a morantel sustained release bolus. *Vet Rec* 108(25):532-535, 1981.
2. Barnes EH, Dobson RJ, Stein PA, LeJambre LF, Lenane LJ: Selection of different genotype larvae and adult worms for anthelmintic resistance by persistent and short-acting avermectin- and moxidectin-selected strains. *Int J Parasitol* 31:720-727, 2001.
3. Bisset SA: Efficacy of a topical formulation of ivermectin against naturally acquired gastro-intestinal nematodes in weaner cattle. *New Zealand Vet J* 38:4-6, 1990.
4. Blackburn BL, Hanrahan LA, Hendrix CM, Lindsay DS: Evaluation of three formulations of fenbendazole (10% suspension, 0.5% pellets and 20% premix) against nematode infections in cattle. *Am J Vet Res* 47:534-536, 1986.
5. Bliss DH, Todd AC: Milk production by Wisconsin dairy cattle after deworming with Baymix®. *Veterinary Medicine / Small Animal Clinician* (September) 1034-1038, 1973.
6. Bliss DH, Todd AC: Milk losses in dairy cows after exposure to infective trichostrongylid larvae: *Veterinary Medicine / Small Animal Clinician*, 1612-1617 (October) 1977.
7. Bliss DH: The *Cattle Producer's Handbook for Strategic Parasite Control*. Somerville, NJ, Hoechst-Roussel Agri-Vet Company, 1988.
8. Bliss DH, Newby TJ: Efficacy of the morantel sustained-release bolus in grazing cattle in North America. *J Am Vet Med Assn* 192:177-181, 1988.
9. Bliss DH, Campbell J, Corwin RM, Kvasnicka W, Laurence L, Strickland J, Whittier D: Strategic Deworming of Cattle (Parts 1-3), Roundtable Discussion. *Agri-Pract* 14(5):34-41, (6):32-37, (7):18-27, 1993.
10. Bliss DH: The worm egg shedding profile of ivermectin pour-on. Technical Bulletin, Hoechst Roussel Agri-Vet Co, Somerville, NJ, 1993.

11. Bliss DH, KvasnickaWG: The fecal exam: a missing link in food animal practice. *Compend Cont Ed Pract Vet* April; 104-109, 1997.
12. Bliss DH, KvasnickaWG: Failure of avermectins to control an outbreak of parasitic gastro-enteritis in a cow/calf herd. In: *Proc 49th Am Assoc Vet Parasitol*. Philadelphia, PA, July 24-28 (Abstract 42), 2004.
13. Bumgarner SC, Brauer MA, Corwin RM, Thomas EA, Myers GH, Strategic deworming for spring-calving beef cow/calf herds. *Am J Vet Res* 189:427-431, 1986.
14. Campbell WC, Benz GW: Ivermectin: a review of efficacy and safety. *J Vet Pharmacol Therap* 7:1-16, 1984.
15. Coles GC, Jackson F, Pomroy WE, Prichard RK, Samson-Himmelstjerma G von, Slivestre A, Taylor MA, Vercruysse J: The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 136:167-185, 2006.
16. Gasbarre LC, Smith LL, Lichtenfels JR, Pilitt PA: The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US. In: *Proc 49th American Assoc Vet Parasitol*. Phildelphia, PA, July 24-28 (Abstract 44), 2004.
17. Gaynard V, Valvinerie M, Toutain PL: Comparison of persistent anthelmintic efficacy of doramectin and ivermectin pour-on formulation in cattle. *Vet Parasitol* 81:47-55, 1999.
18. Hart K, Bliss DH: Efficacy of macrocyclic lactone pour-on under field conditions. In Proceedings of: *Proc 51st Am Assoc Vet Parasitol*. Honolulu, HI, July 15-18 (Abstract 60), 2006.
19. Hooke FC, Clement D, Dell'Osa, Porter RM, MacColl D, Rew RS: Therapeutic and protective efficacy of doramectin injectable against gastrointestinal nematodes in cattle in New Zealand: A comparison with moxidectin and ivermectin pour-on formulations. *Vet Parasitol* 7:43-51, 1997.
20. Hoover RC, Lincoln SD, Newby TJ, Bliss DH: Controlling parasitic gastro-enteritis in pastured cattle. *Vet Med*, August: 1082-1086, 1984.
21. Jacobs DE, Fox MT, Walker MJ, Jones RM, Bliss DH: Field evaluation of a new method for the prophylaxis of parasitic gastroenteritis in calves. *Vet Rec* 108:274-251, 1981.
22. Jones RM: A field study of the morantel sustained release bolus in the seasonal control of parasitic gastroenteritis in grazing calves. *Vet Parasitol* 8:237-245, 1981.
23. Keith E A: Utilizing feed-grade formulations of fenbendazole for cattle. *Agri-Pract – Parasitology* 13 (Jan), 1992.
24. Kvasnicka WG, Krysl LJ, Torell RC, Bliss DH: Cow/calf herd investigation: fenbendazole in a strategic deworming program. *The Compendium*, Food Animal Parasitology, April, 18:113-177, 1996.
25. Kvasnicka WG, Bliss DH, Torrel RC: Evaluation of anthelmintic treatments in cattle grazing Great Basin rangeland in Nevada and California. In: *Proc 42nd Am Assoc Vet Parasitol*. Reno, NV, July 12-22 (Abstract 100), 1997.
26. Kvasnicka WG, Bliss DH: Field efficacy of endectocide pour-on formulations against gastrointestinal nematodes. In: *Proceedings of the 47th Am Assoc Vet Parasitol*. Nashville, TN, July 13-16, 2002.
27. Lawrence JD, Ibarburu MA: Economic Analysis of Pharmaceutical Technologies in Modern Beef Production. Ames, Iowa State University, 2006, pp 1-16.
28. Majia MF, Fernandez Igartua BM, Schmidt EE, Cabaret J: Multispecies and multiple anthelmintic resistance on cattle nematodes in a farm in Argentina: the beginning of high resistance? *Vet Res* 34:461-467, 2003.
29. Prichard RK, Hall CA, Kelly JD, Martin ICA, Donald AD: The problem of anthelmintic resistance in nematodes. *Aus Vet J* 56:239-250, 1980.
30. Prosl H, Superer R, Jones RM, Lockwood PW, Bliss DH: Morantel sustained release bolus: a new approach for the control of trichostrongylosis in Austrian Cattle. *Vet Parasit* 12:239-250, 1983.
31. Raynaud JB, Jones R M, Bliss DH, LeStang LP, Kerboeuf D: The control of parasitic gastroenteritis of grazing cattle in Normandy, France, using the morantel sustained release bolus. *Vet Parasitol* 12:261-272, 1983.
32. Smith LL, Gasbarre LC: The development of cattle nematode parasites resistant to multiple classes of anthelmintic in a commercial cattle population in the US. In: *Proc 49th Am Assoc Vet Parasitol*. Phildelphia, PA, July 24-28 (Abstract 43), 2004.
33. Smith RA, Rogers KC, Husae S, Wray MI, Brandt RT, Hutcheson JP, Nichols WT, Taylor FT, Raines JR, McCauley CT, Pasture deworming and (or) subsequent feedlot performance with fenbendazole. I. Effects on grazing performance, feedlot performance and carcass traits in yearling steers. *Bov Pract* 34:104-114, 2000.
34. Stromberg BE, Vatthauer RJ, Schlotthauer JC, Myers GH, Haggard DL, King VL, Hanke H: Production responses following strategic parasite control in a beef cow/calf herd. *Vet Parasitol* 68:315-322, 1997.
35. Stromberg B, Newcomb H, Bliss D, Hart K, Miller J, Gasbarre L, Craig T, Laurence L: Proposed standardized testing for anthelmintic resistance determination. In: *Proc 52nd Am Assoc Vet Parasitol*. Washington, DC, July 14-17, 2007.
36. Williams JC, Loyacano AF, Broussard SD, Coombs DF, DeRosa A, Bliss DH: Efficacy of a spring strategic fenbendazole treatment program to reduce numbers of *Ostertagia ostertagi* inhibited larvae in beef stocker cattle. *Vet Parasitol* 59:127-137, 1995.
37. Williams JC, Loyacano AF, DeRosa A, Gurie J, Clymer BC, Guerino F: Comparison of persistent anthelmintic efficacy of topical formulations of doramectin, ivermectin, eprinomectin and moxidectin against naturally acquired nematode infection of beef calves. *Vet Parasitol* 85:277-288, 1999.
38. Woods IB, Amaral NK, Bairden K, Duncan JK, Kassai T, Malone JB, Pankavich JA, Reinecke RK, Slocombe O, Taylor SM, Vercruysse J: World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluation the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Vet Parasistol* 58:181-213, 1995.