ORAL PHARMACOKINETIC AND CLINICAL EFFICACY PROFILES OF FENBENDAZOLE IN LLAMAS, SOUTH AMERICAN CAMELIDS

By

ERNEST BEIER III

Doctor of Veterinary Medicine

Oklahoma State University

Stillwater, Oklahoma

1996

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1999

ORAL PHARMACOKINETIC AND CLINICAL EFFICACY PROFILES OF FENBENDAZOLE IN LLAMAS, SOUTH AMERICAN CAMELIDS

Thesis Approved:

Subhash Sangiah

Thesis adviser

Terry W. Lehenbauer

Douglas Carl Fox

Wayne B. Powell

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to thank Dr. Subbiah Sangiah, my major advisor for his unending support and guidance during the course of my graduate training and education. I would like to express my sincere thanks to Drs. Terry Lehenbaurer and Carl Fox for their participation in my committee, their help and guidance. In addition I would like to thank my good friends Dr. Russell and Gaye Ann Higbee for their support, assistance and having an open door for me in my many trips to finish this project.

I would like to extend special thanks to Dr. Thomas Thedford for his support and gracious use of his llamas in these studies.

I proud to have met members of the Oklahoma Llama Breeders Association and grateful for their financial support, Wilber and Betty Holbrook, Edmond, R. L. and Sally Livermore, Elk City, Basil Morton, Orlando, Gar and Ann Graham, Edmond, for their Oklahoma Hospitality, time and use of their animals, in this study

Last but never least, I would like to thank my daughter Meghan, the light of my life, who stood by me and participated in these projects from beginning to end

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

Introduction

South American Camelids (SAC's)

The genus of *Camelus* has evolved and speciated into, *Lamini* and *Camelini* in North America during the Eocene epoch some 40-50 million years ago (Fowler, 1998) During the Pleistocene epoch, the *Lamini* has further evolved into the modern day South American camelids (SAC's) and migrated throughout the western hemisphere However, they became extinct in the later part of the century on the North American continent (Fowler, 1998). The *Camelini*, "old world camelids" migrated to Eurasia over the Bering land bridge during the Pleistocene epoch, where they later became domesticated (Fowler, 1998). The (SAC's) were domesticated around 4000 BC, and became a main stay of the Inca empire, providing food, clothing, and transportation (Fowler, 1998, Hoffman, 1996)

During the nineteenth century, SAC's were exported to other countries of the world as zoo animals. This exportation ended at the turn of the century when all of the Andean countries including Peru and Bolivia with portions of Argentina, Chile, Colombia, and Ecuador prohibited the export of these animals. In the 1980's, the ban on

exportation was lifted for alpacas (*Lama pacos*) and llamas (*Lama glama*), by the countries containing the Andes mountains including Peru, and Bolivia Presently, imports of SAC's into the U S.A are limited by public health concerns, such as the spread of Foot and Mouth Disease (Fowler, 1998). The number of Llamas (*Lama glama*) in North America is currently around 100,000 with approximately 10,000 Alpacas(*Lama pacos*), and smaller numbers of vicuña(*Vicugna vicugna*) and guanaco (*Lama guanicoe*) (personal communication). There are about 1500 Llamas raised by at least 50 farms in Oklahoma (Oklahoma Breeders Association, 1998).

The General Biology of SAC's

Llamas are one of the four species known as New world camelids which inhabit the Andean countries, the other being the alpaca, the guanaco, and the vicuña. The four members of the genus Llama are so closely related, that they all produce fertile hybrids when crossbred. Llamas and alpacas exist only as domestic species. Vicuñas and guanacos are the only two species that can still be found in the wild (Fowler, 1998) The Llama was bred specifically to produce a large, strong animal for packing function, while the alpaca was bred to accentuate its natural finer wool (Hoffman, 1996).

Obviously modern transportation has reduced the importance of the llama as beast of burden. Primary emphasis is now being placed on this animal primarily as a source of food and secondarily as fiber (Fowler, 1998).

The SAC's including Llamas and alpacas are induced ovulators, stimulated by copulation with their respective males. Llamas have an 11 month gestation period (Fowler, 1998).

The Gastrointestinal System; its Anatomy and Physiology

SAC's including llamas are modified herbivorous ruminants, and ungulates with some major anatomical and physiological differences. The SAC's having evolved during the arid Pleistocene epoch have developed specific adaptations for dealing with dehydration and extremes in temperature.

Ruminants have a four chambered stomach (Figure 1); the rumen, the first chamber receives food when first ingested.

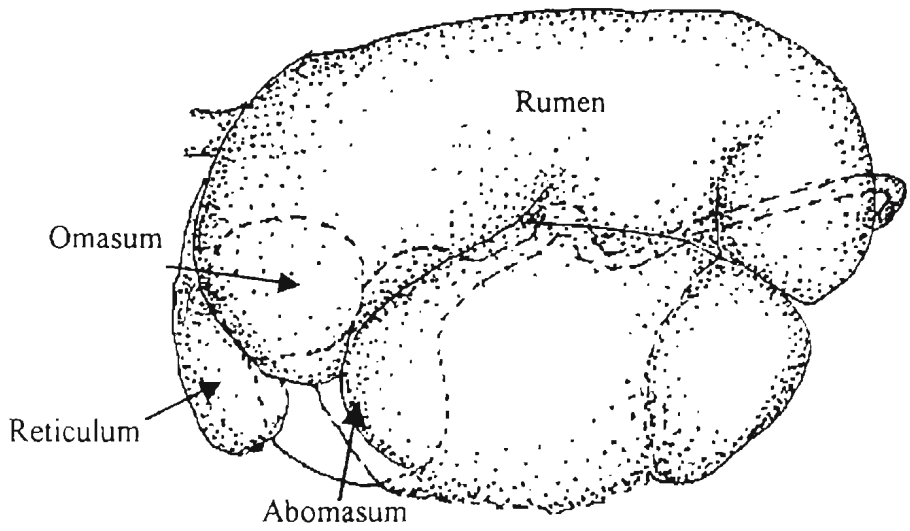


Figure 1. Anatomy of the Ruminant Stomach. (after Dice, 1987)

The reticulum which is closely associated in form and function with the rumen, has been combined the two chambers functionally into the rumenorecticulum by many researchers. The symbiotic flora of the ruminoreticulum ferments normally indigestible plant material. The rumen contains four distinct layers of stratification; the gas layer, the fibrous mat

layer, the liquid layer and fine particulate layer. Volatile fatty acids produced by the flora accumulate into the gas layer, are absorbed by the walls of the rumen and eructated to be absorbed through the lungs. The fibrous material from this stomach is regurgitated and chewed as a cud by the ruminant when resting to reduce the size of the particles providing a larger surface area for the rumen flora to digest. The liquid and particulate layers provide a media for the flora to be biologically functional. The fine particulate material is then moved into the omasum, the third compartment Digestion continues in this compartment through grinding of plant material into still finer particles while squeezing the ingesta to remove excess water In the abomasum, the glandular stomach of the ruminant completes digestion through acidification and enzymatic action. The ingested food then passes on to the small intestine, for absorption of nutrients into systemic circulation. The total length of both small and large intestine is approximately 50 m in ruminants (Dice, 1987).

The unique characteristics of the SAC's stomach include ill defined pillars and columns SAC's have a three chambered stomach: C-1, C-2, and C-3 (Figure 2). Llamas lack the stratification of ingesta, analogous to that of the ruminants and do not accumulate gas Volatile fatty acids are absorbed rapidly from the C-1 stomach at a rate 2-3 times greater than sheep These animals can recycle the urea from the blood stream through the stomach, allowing symbiotic flora to utilize it as a nitrogen source for protein production. This enables SAC's to extract more protein and energy from poor quality forage than their cousins, the ruminants. The ingesta of C-3 is relatively dry compared to that of a ruminant, due to the absorption of the water in the proximal 4/5 of the stomach. The terminal 1/5 of the C-3 stomach contains the gastric glands. The total

length of SAC's small intestine is 11.5-12.0 m with 1, 9.5–10.0, and 1.0 m in lengths of duodenum, jejunum and ileum respectively. The large colon is 7.5 m with the caecum 10 cm in length and the small colon of 6.0 m in length (Fowler, 1998).

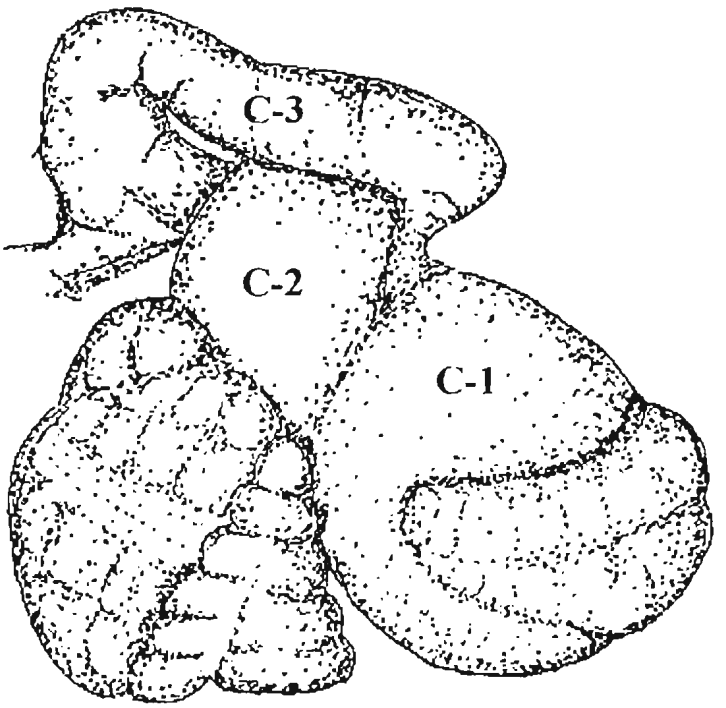


Figure 2. Anatomy of the South American Camelid Stomach.

Gastrointestinal Parasitism in Llamas

All animals, including llamas, are host to a number of external and internal parasites (Fowler, 1998; Johnson, 1994). Many llama owners feel that these animals are parasite free due to the fact that these animals defecate on dung piles rather than over the entire pasture like ruminants (Johnson, 1994). Most of the published work on the incidence of parasitism are found in articles from South American countries, with limited

work having been done in North America. There is accumulating evidence to indicate that gastrointestinal parasites are significant disease producers in llamas. Studies conducted in South American countries have demonstrated the presence of Trichostrongyloidea, *Nematodirus*, and Trichuroidea (*Trichuris*, and *Capillaria)* eggs in llamas fecal materials Similar studies conducted in Oregon have confirmed these findings. Trichostrongyle like eggs are produced by *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, and Strongyles (*Bunostomum*, and *Oesophagostomum)*, all of which are reported to occur in llamas as well as cattle, sheep, goat, and some wild ruminants in North America. Additional evidence indicates the presence of gastrointestinal Trichostrongylidae including *Camelstrongylus*, *Spiculopteragia*, *Lamenema*, and *Graphinema* (Chenney, 1989)

The clinical signs of gastrointestinal parasitism in llamas are very similar to those of parasitized cattle and sheep The parasitized llamas do not grow or mature as rapidly as nonparasitized animals Overt clinical signs seen in llamas with heavy gastrointestinal parasites infestation are diarrhea, dehydration, emaciation, hypoproteinemia, and anemia. Most animals, however, will only show only sub-clinical effects of parasitism; depressed growth rate, poor feed conversion, and depressed milk production (Rickard, 1991).

Any condition that will reduce the animal's natural health including, stress, certain vaccinations, birthing, inadequate nutrition, vitamin deficiencies and genetic predisposition for infection will break down the animals natural resistance to parasites. Extremely large parasite challenges can break down and overwhelm the natural resistance factors and cause clinical disease in llamas (Lapage, 1962)

Parasitism and the Need for Chemical Control of Parasites

In the wild populations of mammals, exposure to internal parasites was naturally kept to a minimum through herd movements (Schmidt, 1981). Additionally, through continuous low levels of parasite infestation, animals were able to mount an immune response to these parasites keeping infection at a low level. Through domestication of ungulates, man has placed restrictions on the free movement of these animals, disrupting the balance of natural controls that evolved over a long period of time (Cheng, 1986).

Problems presented in the control of internal parasites include: exquisite adaptation to the animals they infect Parasitic helminths generally elicit a very weak initial immune responses from the host with gradually increasing resistance to infection after 4-6 months of exposure and resistance can be lost again in as little as 4 weeks (Cheng, 1986). Another problem in control of intestinal parasites involves their eggs that may survive harsh environmental conditions for extended periods of time and retain their viability (Schmidt, 1981).

Chemical control does not totally eliminate helminth infestation: it only keeps the numbers of parasites to a level that the animal can reasonably tolerate. Long term usage must be balanced with the disadvantages of selecting for drug resistance in parasites being controlled, as has seen in sheep (Roberson, 1988). Older anthelmintic compounds, including cupric sulfate, arsenic compounds and alkaloids such as nicotine, were used with limited success and had very narrow margins of safety (Roberson, 1988; Barragry, 1994).

Chemical Control of Internal Parasites in SAC's

The goal for controlling internal parasites by the use of chemical compounds is to eliminate the parasites while not causing harm to the host species. The classes of compounds currently used in SAC's include drugs and toxins that interfere with the parasites neuromuscular junctional transmission, energy metabolism, and transport of energy containing molecules.

Phenothiazine, can be classified as a toxin. It was first synthesized in 1885 and identified as an anthelmintic in the 1930s. It was extensively used as an anthelmintic until the 1960s as the primary parasitic control method in ruminant livestock In the 1960's, it was discovered that parasites had developed resistance to this drug. A side effect of phenothiazine is that it caused the urine and milk to be pink in color for several days after administration. Animals would become stained by the urine on the wool and skin which reduced the value of each of those products. Phenothiazine is currently used at low levels as a feed additive for continuous parasite control (Roberson, 1988).

Other drugs with a wider therapeutic index were discovered and developed for the livestock industry. Praziquantel, a diethylenediamine causes hyperpolarization of the muscle membranes of the cestode parasite resulting in paralysis and death. Praziquantel had no effect upon nematodes (Barragry, 1994). An imidazole (Levamisole) were first marketed in 1965, pyridines (methyluridine) and tetrahydropyrimidines (pyrantel) were marketed in 1966 (Barragry, 1994) Imidazoles and pyridines act by binding irreversibly with acetylcholine (Ach) receptors. They block conduction of nerve impulses across the neuromuscular synapses paralyzing the parasites. (Roberson, 1988; Barragry, 1994).

Ivermectin (Ivomec), an avermectin introduced in 1981, increases the release of gamma-aminobutyric acid (GABA) from the synaptic nerve endings. This hyperpolarizes the normal resting potential of post-synaptic nerve cells, inhibiting normal nerve transmission and causing a flaccid paralysis of muscles of the nematodes expelling them from the host (Roberson, 1988; Barragry, 1994).

A third group of anthelmintic drugs includes, benzimidazoles, substituted phenols and salicylanilides that interfere with energy metabolism and/or the transport of energy containing molecules.

The salicylanilides (clioxanide) and substituted phenols (clorsulon) uncouple the electron transport system of cestodes and trematodes respectively, preventing the oxidative phosphorlization of high energy molecules through interruption of the Krebs cycle reactions. These particular classes of drugs act better on cestodes and trematodes than nematodes possibly due to poor uptake of the drugs by the nematodes (Roberson, 1988, Barragry, 1994).

Benzimidazoles as Anthelmintics

Benzimidazoles are a class of drugs that fit the requirements for low toxicity, a high degree of efficacy, numerous efficacious analogues and have the ability to vary routes of administration for treatments. Thiabendazole, the parent compound of this group, was discovered, developed, and then licensed for use in the 1961. Currently benzimidazoles are utilized world wide on humans, cattle, sheep, goats, pigs, horses, birds, and exotic wild animals. Since the development of thiabendazole, several hundred compounds have been synthesized, based on the core structure of the compound. Of all

the benzimidazole analogues, ten different drugs including albendazole, cambendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, parbendaxole, thiabendazole, and thiophanate have been approved for use as anthelmintics.(Roberson, 1988)

The chemistry of the benzimidazoles is based upon substitutions to the 1,2-diaminobenzene ring system with substitutions occurring in the number 2 and 5 ring positions. Thiabendazole is being phased out of human medicine due to its relativity high toxic effects. At the same time, other members of this group are widely used against a variety of gastrointestinal helminths (Roberson, 1988, Mckellar, 1990, DeSilva, 1997; Barragry, 1994).

With the exception of thiabendazole, albendazole, and oxfendazole, all other members of this compound group exhibited limited absorption from the gastrointestinal tract due to their limited solubility. Thiabendazole, flubendazole and mebendazole are rapidly absorbed, with peak plasma concentrations occurring within 2-7 hrs of administration showing variation dependent upon the species of animals treated. Albendazole, fenbendazole, oxibendazole, parbendaxole, and thiophanate reach peak plasma concentrations in 6-30 hrs after administration, with concentrations never more than 1% of the administered dose (Roberson, 1988). Albendazole, cambendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, parbendaxole, thiabendazole and thiophanate are insoluble or only slightly soluble in water. Albendazole, cambendazole, oxfendazole, and parbendaxole are soluble in alcohol. Thiabendazole is only slightly soluble in alcohol. Flubendazole, and mebendazole are soluble in formic acid

Fenbendazole is soluble in dimethylsulfoxide. Thiophanate is soluble in cyclohexanone (Roberson, 1988)

Absorption of benzimidazoles from the gastrointestinal tract is greater in monogastric than in the ruminant animals Many benzimidazoles are poorly absorbed and excreted unchanged in the feces within the first 48 hrs. However, small amounts of the drugs can be detected up to 11 days after oral administration in the urine and feces. Ruminants, retain much of the benzimidazole in the rumen rather than passing it into the abomasum. This increases the drug's retention time, increasing absorption into the blood stream which in turn augments the antihelmintic activity (Hennessy and Prichard, 1981). The remaining absorbed drug is excreted in the urine with only a small portion of the drug excreted as the metabolites. The drug is metabolized by the liver via the cytochrome P-450 system and microsomal flavin monooxygenases (Gottschall, 1990). Residual quantities of the drugs have been detected in the liver up to 2 wks after administration. Therefore, depending upon the formulation of the drug used, all benzimidazoles require a minimum withdrawal time of 5-10 days; one needs to refer to label instructions for withdrawal times in specific species (Roberson, 1988).

Anthelmintic activity of benzimidazoles is produced by interference of glucose metabolism. Interference is accomplished in several ways, the main mode of action of the benzimidazoles is to bind with the microtubule structural sub units, tubulin, which in affects the microtubules, preventing further development of these structures. Without the microtubules, the organism cannot carry on normal metabolic functions and the organism dies. The benzimidazoles attach selectively to the form of tubulin that predominates in the parasites and is not found in the mammalian hosts. Resistance to the

drugs by the parasites is a function of selection for those parasites that can form tubulin that has the same form as the host animals (Lacey, 1990; Mckellar, 1990).

Additionally, benzimidazoles have been shown to interfere with the fumarate reductase which inhibits the generation of ATP in the mitochondria causing the parasite to die due to a lack of usable energy (Roberson, 1988).

Toxicity of Benzimidazole Anthelmintics

Most of the members of this class of drugs, except albendazole, mebendazole and thiabendazole, have a wide therapeutic index, and high doses of the drugs are well tolerated by young, old, sick and debilitated animals. In humans, thiabendazole has been shown to rarely cause anorexia, nausea, vomiting, dizziness, diarrhea and occasionally a fever. Thiabendazole does have a hepatotoxic potential, therefore should be used with caution in animals with liver disease. Mebendazole has been shown to be teratogenic and embryotoxic in laboratory animals at a single dose. Dogs treated with mebendazole at therapeutic levels occasionally develop acute hepatitis and jaundice. Albendazole has been shown to have a teratogenic effect in sheep, rabbits and rats; therefore, its use during pregnancy is contraindicated Cattle have not shown any embryotoxic or teratogenic effects as seen in other species that have been treated with albendazole, however, poor conception rates have been reported in treated animals. It should also be used with caution in patients with hepatic disease (Roberson, 1988).

Parbendazole and cambendazole exert a teratogenic effect in pregnant ewes during the time of limb formation of the fetus, around day 20 of the pregnancy.

Therefore, they should not be used until after the 4th week of pregnancy of the ewe (Roberson, 1988)

Fenbendazole as Anthelmintic

Fenbendazole is marketed in the United States by Hoechst-Roussel Agri Vet It has several trade names including, Panacur®, Safe-Guard® and Axilur®. Panacur® is the veterinarian-labeled drug and is marketed in the following formulations, 22.% granules for horses and dogs, 10% suspension for horses and cattle, a 10% paste for horses and cattle

Safeguard® is the over-the-counter label of fenbendazole, marketed by Hoechst-Roussel Agri Vet Co Safeguard® is marketed in the following forms, as a medicated 0.1% supplement block and medicated 20% protein block for cattle., as a free choice medicated mineral mix and a 0.5% top dressing for cattle. It is in a 1.8% granular formulation for hogs. The dosage for cattle and horses is 5 mg/kg. of fenbendazole for one treatment Dogs require a treatment of 50 mg/kg for three days.

Fenbendazole in cattle is effective against the lung worm, *Dictyocaulus viviparus*, the stomach worms, *Haemonchus contortus*, *Ostertagia ostertagi*, *Trichostrongylus axei* and the intestinal worms, *Bunostomum phlebotomum*, *Nematodirus helvetianus*, *Cooperia punctata*, *C. oncophora*, *Trichostrongylus axei*, and *Oesophagostomum* sp. (Kanzler, 1995).

Anthelmintics in Llamas

There are no anthelmintics approved for use in SAC's in the United States. There are, however, many anthelmintics administered to these animals in an extra label fashion based upon the veterinarians best clinical judgment.

TABLE I

ANTHELMINTICS CURRENTLY ADMINISTERED AS EXTRA LABEL DRUGS TO SOUTH AMERICAN CAMELIDS (JOHNSON, 1994)

Fenbendazole	Panacur®	Hoechst-Roussel Agri vet
Ivermectin	Ivomec®	Merck Agri vet
Levamisole	Tramasole®	Merck Agri vet
Mebendazole	Telmin®	Pitman -Moore Inc.
Pyrantel pamoate	Strongid®	Pfizer Inc.
Thiabendazole	Equizole®	Merck Agri vet
Praziquantel	Droncit®	Haver-Lockhart Labs
Clorsuon	Curatrem®	Merck Agri vet

Internal parasites have been identified as a major health problem in the livestock industry. Although there are significant numbers of "minor species" animals raised in this country for food, fiber and companion purposes, there is lack of legal (FDA-CVM approved) drugs for use in these animals. There are over 100,000 llamas (*Lama glama*),

and 10,000 alpacas with smaller numbers of guanacos and vicunas in the United States by current estimates of the llama breeder association. All members of the genus *Lama* have the same number of chromosomes; therefore, the physiology of these animals will be the same. Additionally the cash values of llamas range from $800.00 to $50,000 00 and alpacas even higher (personal communication). In Oklahoma alone, there are 45 breeding farms that are members of the Oklahoma Llama Association, with numerous others that are not registered with the association (Oklahoma Llama Association, 1998). Although the incidence of intestinal parasitic diseases and their impact on the health of SAC's in currently unknown, there is a significant need for identifying an anthelmintic agent that is clinically effective and safe for use in these animals. Fenbendazole is a member of the benzimidazole group of anthelmintics, and has broad spectrum of activity against gastrointestinal helminths, lungworms, and some tapeworms (Roberson, 1988)

This drug is currently approved for and widely used in horses, dogs, pigs and cattle. Although this drug has been studied extensively in sheep and goats, fenbendazole is currently being recommended and used as an extra label anthelmintic in llamas, alpacas and other SAC's (Fowler, 1998). However, there are no studies concerning the pharmacokinetics, efficacy and safety profiles of this drug in these animals.

In our preliminary clinical trials on fenbendazole (Safe-Guard®) at a dose of 5 mg/kg in 59 llamas throughout the state of Oklahoma, we have found that the drug produces a significant reduction in egg production of Trichostrongyloidea (*Nematodirus* and *Trichostronghus*), and Trichuridoidea (*Trichuris* and *Capillaria*), by fecal egg count (unpublished data). Therefore, the major objective of this study was to characterize oral pharmacokinetics and clinical efficacy profiles of fenbendazole in llamas (SAC's).

REFERENCES

Barragry, T.B. 1994. *Veterinary Drug Therapy*. Lea and Febiger, Philadelphia, pp 80-118.

Breeder and Service Directory. 1988. Oklahoma Llama Breeder's Association. pp 1-28

Cheney, J.M. and Allen, G.T. 1989. Parasitism in llamas. *Veterinary Clinics of North America: Food Animal Practice* 5(1):217-225.

Cheng, T.C. 1986. *General Parasitology*, 2nd Ed. Academic Press, Orlando. pp 1-97.

DeSilva, N., Guyatt, H. and Bundy, D. 1997. Anthelmintics: a comparative review of their clinical pharmacology. *Drugs* 5:769-788.

Dice, K M., Sack, W.O. and Wensing, C.J. 1987. *Textbook of Veterinary Anatomy*. W.B. Saunders, Philadelphia. pp 649-650.

Fowler, M. 1998. *Medicine and Surgery of South American Camelids*. Iowa State University Press. pp 155-163.

Gottschall, D.W., Theodorides, V.J. and Wang, R. 1990. The metabolism of benzimidazole anthelmintics. *Parasitology Today*. 6(4):115.

Hennessy, D.R. and Pritchard, R.K. 1981 The role of absorbed drug in the efficacy of oxfendazole against gastrointestinal nematodes. *Veterinary Research Communications* 6:45-49

Hoffman, E. 1996. All about alpacas. *Llamas, International Camelid Journal* 10(1) 12-13.

Johnson, L. 1994. Update on llama medicine. *Veterinary Clinics of North America, Food Animal Practice* 10(2):248-257

Kanzler, K. 1995 *Veterinary Pharmaceuticals and Biologicals*, 9th Ed. Veterinary Medicine Publishing Group, Lenexa, KS. pp 403-409.

Lacey, E. 1990. Mode of action of benzimidazoles. *Parasitology Today* 6(4):112-115.

Lapage, G. 1962. *Veterinary Helminthology and Entomology*. Williams and Wilkins Co., Baltimore, Md

McKellar, Q.A. and Scott, E.W. 1990. The benzimidazole anthelmintic agents. *Journal of Pharmacology and Therapy* 13:223-247.

Rickard L.G. and Bishop, J.K. 1991. Helminth parasites of llamas (*Lama glama*) in the Pacific Northwest. *Journal of the Helminthological Society of Washington* 58.110-115

Rickard, L.G. 1994. Parasites. *Veterinary Clinics of North America: Food Animal Practice* 10(2):239-247

Robertson, E.L. 1988. *Veterinary Pharmacology and Theraputics*, 6th Ed. Boothe, N.H. and McDonald, L.E. (Eds). Iowa State University Press, Ames. pp 877-882, 917-919, 1012-1015.

Schmidt, G.D. and Roberts, L. S. 1981. *Foundations of Parasitology*, 2nd Ed. Mosby, St. Louis. pp 4-31.

Soulsby, E.L. 1982. *Helminth Arthropods and Protozoa of Domestic Animals*, 7th Ed. Lea and Febiger, Philadelphia. pp 784-785.

CHAPTER II

ORAL PHARMACOKINETICS OF FENBENDAZOLE IN LLAMAS, SOUTH AMERICAN CAMELIDS

Abstract

Llamas, South American Camelids are increasingly popular in the United States, as a source of fiber, livestock guard, and pack animals. Internal parasites have been identified as a major health problem in the livestock including llama industries. Currently, there are no approved anthelmintics available for use in llamas. In this study, the pharmacokinetics of a single, oral dose of fenbendazole paste at 5 mg/kg was evaluated in llamas. Plasma fenbendazole concentration time profiles were best described by a single compartment model. After oral administration of fenbendazole, the t_{max}, and the C_{max} were 28.39 ± 12.80 h, and 0.27 ± 0.17 μg/ml, respectively The $t_{1/2ka}$ and $t_{1/2\,kel}$ were 16.25 ± 11.67 h and 36.00 ± 25.00 h, respectively. The apparent volume of distribution (Vd) and the area under the curve (AUC) were 11.28 ± 4.66 l/kg, and 22.52 ± 8.67 μg/ml, respectively, with a systemic clearance (Cl_B) of 0.26 ± 0. 11 l/h/kg. The results of this study indicate that fenbendazole when administered orally in the paste formulation, was more rapidly absorbed in llamas compared to other ruminants including sheep, goats and cattle as indicated by the short time taken to reach peak plasma concentrations Higher peak plasma concentrations of fenbendazole found in llamas following oral

administration indicated a more complete absorption of the drug in this species compared to other ruminants including cattle, sheep and goats. It was also found that the rate of elimination of fenbendazole was prolonged in llamas as compared to sheep, goats, and cattle. This may be partly due to slower hepatic metabolism of the drug. This was consistent with the observation that fenbendazole when administered orally at a single dose of 5 mg/kg, was very effective clinically for a period of 4 wks, in llamas with naturally occurring gastrointestinal parasitism (Beier, *et al.*, 1998).

Introduction

Internal parasites have been identified as a major health problem in livestock including llamas. Although there are significant numbers of "minor species" animals raised in this country for food, fiber and companion purposes, there is lack of legal (FDA-CVM approved) drugs for use in these animals. The number of Llamas (*Lama glama*) in North America is currently around 100,000 with approximately 10,000 Alpacas(*Lama pacos*), and smaller numbers of vicuña (*Vicugna vicugna*) and guanaco (*Lama guanicoe*) (personal communication). All members of the genus Llama have the same number of chromosomes; therefore, the physiology of these animals will be the same. In Oklahoma alone, there are 45 breeding farms that are members of the Oklahoma Llama Breeders Association, with numerous others that are not registered with the association (Oklahoma Llama Breeders Association, 1998). Although little is known concerning the incidence of significant intestinal parasitic diseases and their impact on the health of South American Camelids (SACs), there is a significant need for identifying an anthelmintic agent that is clinically effective and safe for use in these

animals. Fenbendazole is a member of the benzimidazole group of anthelmintics, and has a broad spectrum of activity against gastrointestinal parasites, lungworms, and some tapeworms. This drug is currently approved and widely used in horses, dogs, pigs and cattle (Roberson, 1988)

Fenbendazole has been studied extensively in sheep and goats, and is currently being recommended and used as an extra label anthelmintic in llamas, alpacas and other SAC's (Fowler, 1998; Cheney, 1989). However, there are no studies available concerning the pharmacokinetic, clinical efficacy and safety profiles of this drug in these animals. Therefore, the major objective of this study was to characterize oral pharmacokinetic profiles of fenbendazole in llamas.

Materials and Methods

Animals

Five healthy, young adult llamas of both sexes purchased from local farms were used in this study. Each animal's health was assessed by physical examination, CBC, blood chemistry profiles, urinalysis, and fecal egg counts. The llamas were individually housed at the University Lab Animal Resources' barn in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH, 1985). Drinking water and a balanced ration of grain and prairie hay were provided ad libitum.

Experimental Procedure

Oral Administration of Fenbendazole and Collection of Blood Samples

A group of 5 llamas were used in this experiment Using chute restraint, aseptic surgical technique and a local anesthetic, indwelling catheters were placed in the right jugular vein of individual llamas. Twenty-four hours later, at time 0, fenbendazole (Safe Guard™ [paste], Hoechst Roussel Agri Vet, Somerville, NJ) at a dose of 5 mg/kg was orally administered to each animal using a dispensing gun (Hoechst Roussel Agri Vet, Somerville, NJ). Five ml blood samples were collected in a heparinized syringe via the indwelling intravenous catheter at 0, 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 120 and 144 hr periods. Following sample collection, 5 ml of normal saline was injected to replace the volume of the blood sample collected. Blood samples were centrifuged at 2000 X g for 10 minutes; plasma samples were collected and stored at -20°C until analysis

Chemicals

All reagents used in this study were HPLC grade. Deionized distilled water (Milli-Q water system, Millipore Corp., Bradford, MA) was used throughout the study Analytical (HPLC) grade methanol, acetonitrile, chloroform, and ammonium phosphate were obtained from Fisher Chemicals (Fair Lawn, NJ).

Standard Solutions.

Stock solutions of fenbendazole and its internal standard, oxibendazole (Sigma

Chemical Co., St. Louis, MO), were prepared in methanol at concentrations of 0.25 mg/ml and 0.1 mg/ml, respectively, and then stored at -20°C. The stock solutions were diluted further to yield working solutions for the preparation of HPLC standards as needed.

Extraction Procedure

For determination of fenbendazole, 10 (μg/ml) (l of oxibendazole) was added to 500 (μl of plasma) as the internal standard Seven ml of chloroform was then added, and the mixture was vortexed thoroughly for 3 min. Following centrifugation (2000 X g, 5 min), the organic phase was transferred into a clean test tube after the aqueous layer was frozen under acetone/dry ice conditions. The chloroform extracts were evaporated to dryness under a slow steady stream of nitrogen. The resulting residue was reconstituted in 100 (μl of methanol) and an aliquot of 50 (μl of this solution) was injected into the HPLC for analysis. The extraction recovery of fenbendazole and oxybendazole from llamas plasma in this study were 68.4 (3.31%) and 68.4 (1.67%), respectively.

HPLC Determination of Plasma Fenbendazole

The HPLC analysis of plasma fenbendazole was carried out using the Waters HPLC System (Waters, Millipore Co., Milford, MA). This unit was comprised of a pump (Model 501), a tunable absorption detector (Waters 484) and chromatographic software (Maxima 820). The analytical Lichrospher 100, RP-18 column (5 m, 250 x 4 mm) (Hewlett Packard Inc.) and a guard column, Lichrospher 100, RP-18 (5 m, 4 x 4 mm)

(Hewlett Packard Inc.) were used to resolve fenbendazole and oxibendazole (internal standard), respectively

The mobile phase acetonitrile: 10 mM ammonium phosphate buffer (pH 3 7, 48 52, v/v) degassed, using a vacuum bottle and pump at -15 psi was used. The column was equilibrated and eluted under isocratic conditions utilizing a flow rate of 1.0 ml/min at ambient temperature. The detection wavelength was set at 292 nm. Peak width, response time and slit were set a >0.03 min, 0.5 s and 8 nm, respectively. Standard curves were prepared daily by comparing peak area ratios of fenbendazole concentrations in plasma to peak area ratios of the internal standard

Method validation was performed by comparing peak area ratios of fenbendazole oxybendazole (internal standard) from a range of standard solutions in drug-free llama plasma. Additionally, precision was determined by comparing the coefficients of variation of inter-day variations in the peak area ratios of the standard curves. The results of the variations indicated that the HPLC method was suitable for pharmacokinetic studies of fenbendazole in llamas with an accuracy of 94 ± 8%, coefficient of variation of 10% and detection limit of quantitation of 0.01 μg/ml.

Determination of the Pharmacokinetic Parameters
of Orally Administered Fenbendazole

Data fitting and pharmacokinetic parameter calculations were carried out by estimation using the Boomer program (Bourne, *et al.*, 1984), and by analysis using the PharmK program (Lu and Mao, 1993). An appropriate pharmacokinetic model was chosen on the basis of lowest weighted squared residuals, lowest Akaike's information criterion (AIC) value, R-squared, and correlation coefficient. The area under the curve

(AUC) was calculated by the trapezoidal rule between 0 hr and the last sampling time plus C/k, where C is the concentration of the last sampling and k is the elimination rate constant (Gibaldi *et al.*, 1975). The systemic clearance (Cl_B) was determined by dividing the dose by the AUC. The time (t_{max}) taken to achieve peak concentration (C_{max}) was calculated using differential calculus (Gibaldi *et al.*, 1975). The results are presented as means and standard errors of the mean (SEM).

Results and Discussion

Several combinations of acetonitrile: 10 mM ammonium phosphate buffer (pH 3.7) were evaluated as possible mobile phases. It was determined that a ratio of acetonitrile: 10 mM ammonium phosphate buffer (pH 3.7, 48:52, v/v) was most suitable for separation of fenbendazole. Changing the ratios of acetonitrile and ammonium phosphate buffer alters the retention time of the fenbendazole. Larger areas yielded shorter retention time, which caused interference by other peaks produced by the methanol solvent.

Oral Pharmacokinetic Parameters

The plasma fenbendazole concentration- time curves for oral route of administration is presented in (Fig. 1). The concentrations range of plasma was (0.025 - 0.283 μg/ml) after an oral dose of 5 mg/kg fenbendazole. The plasma concentration data for fenbendazole after oral administration of 5 mg/kg was best fitted to the exponential equation as shown below.

$$C = B_1 e^{-k_{el}t} - B_2 e^{-k_a t}$$

The C is plasma fenbendazole concentration; B_1 and B_2 represent mathematical coefficients; and k_{el} and k_a represent the first order elimination and absorption rates respectively. Pharmacokinetic parameters of orally administrated fenbendazole are presented in Table II.

TABLE II

PHARMACOKINETIC PARAMETERS FOLLOWING ORAL ADMINISTRATION OF FENBENDAZOLE PASTE (5 mg/kg) TO LLAMAS (n=5)

Parameter	Value (Mean ± SEM)
I. K_{EL} (H^1)	II. 0.025 ± 0.01
K_a (h^1)	0.070 ± 0.06
$T_{1/2ka}$ (h)	16.25 ± 11.67
$T_{1/2kel}$ (h)	36.000 ± 25.00
Cl_B (l/h/kg)	0.260 ± 0.11
Vd (l/kg)	11.280 ± 4.66
C_{max} (μg/ml)	0.270 ± 0 17
T_{max} (h)	28.390 ± 12.80
AUC (μg • h/ml)	22.520 ± 8.67

Abbreviations: K_{el} - first order elimination rate constant; K_a - first order absorption rate constant; $t_{1/2ka}$ is absorption half- life, K_{el} is elimination half -life; Vd is apparent volume of distribution calculated using AUC; Cl_B - is body clearance of the drug.

Following oral administration of fenbendazole in llamas, the drug reached peak concentration of 0. 27 ± 0 17 µg/ml in the blood at approximately 10 hr and dropped to a concentration below the detectable level at 140 hr (Figure 3)

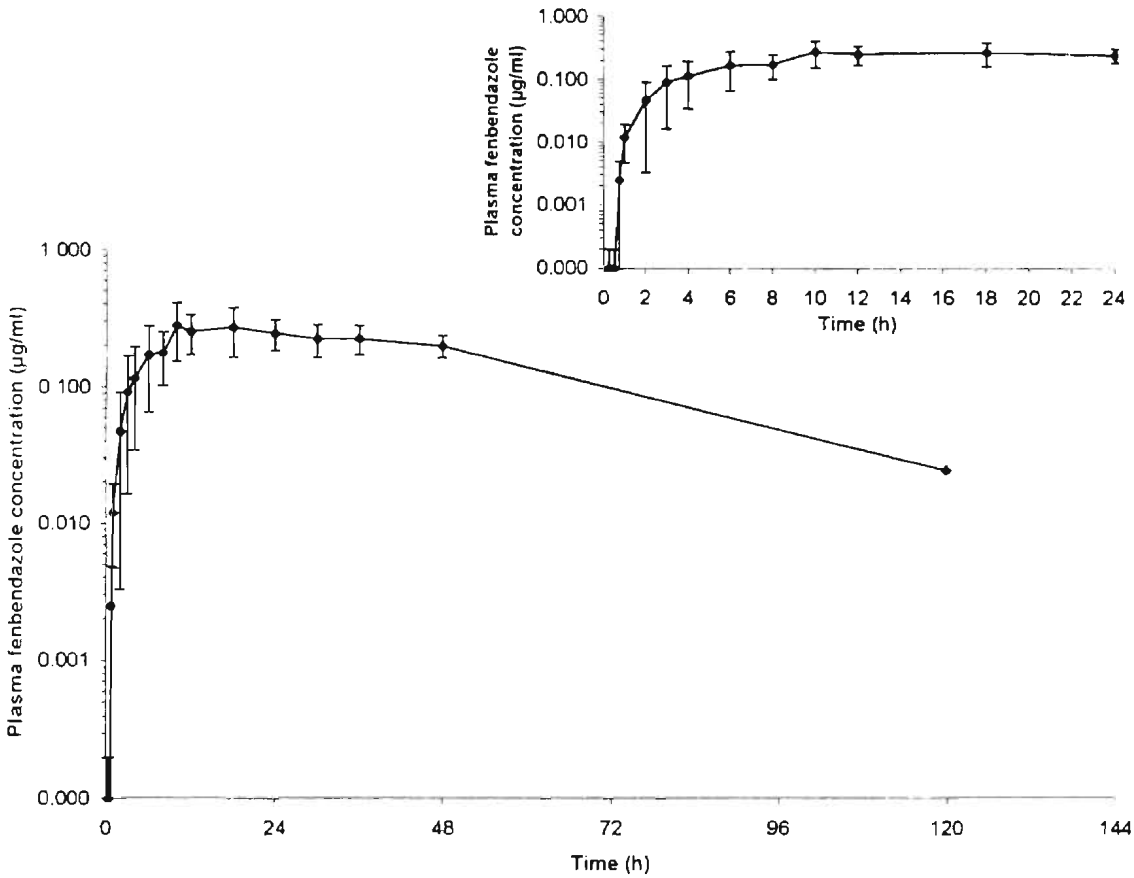


Figure 3. Mean Plasma Concentration Time Curves of Fenbendazole Paste in Llamas (n=5) Following Oral Administration of A Single Dose at 5mg/kg.

The peak plasma concentration (0.28 µg/ml) of fenbendazole in llamas is approximately two-thirds of the reported value (0.40 ug/ml) for sheep (Lanusse *et al.*, 1995). However, it is twice the value of goats (0.13 µg/ml) and cattle (0.15 µg/ml) (Short *et al.*, 1987; Short *et al.*, 1987; Lanusse *et al.*, 1995). The time of 10 hr

necessary to achieve peak plasma concentration in llamas was considerably less than that of sheep (20 hr), goats (24 hr) and cattle (12 hr) (Short *et al.*, *et al.*, 1987; Short *et al.*, 1987; Lanusse *et al.*, 1995). However, the elimination time (120 hr ± 0.25) is significantly longer in llamas compared to those of sheep (70 hr), goats (73 hr) and cattle (96 hr) (Short *et al.*, 1987; Short *et al.*, 1987; Sanyal *et al.*, 1993, Lanusse *et al.*, 1995).

Conclusions

The results of this study indicate that fenbendazole when administered orally in the paste formulation, was more rapidly absorbed in llamas compared to other ruminants including sheep, goats and cattle as indicated by the short time taken to reach peak plasma concentrations. Higher peak plasma concentrations of fenbendazole in llamas than those of goats or cattle indicated a more complete absorption of the drug in this species. However the absorption was less compared to sheep. It was also found that the rate of elimination of fenbendazole was prolonged in llamas compared to sheep, goats, and cattle. This may be partly due to slower hepatic metabolism of the drug. This was consistent with the observation that fenbendazole when administered orally at a single dose of 5 mg/kg, was very effective clinically for a period 4 wks, in llamas with naturally occurring gastrointestinal parasitism (Beier *et al.*, 1999).

Acknowledgements

This study was supported in part by funds from the office of Dr. Tom Thedford, Assistant Dean for Outreach, College of Veterinary Medicine, Oklahoma State

University, Stillwater Oklahoma, OK 74078 and a grant from Oklahoma Llama Breeders Association, Norman, OK 73071.

REFERENCES

Akaike, H. 1973. *Anew Look at the Statistical Model Identification.* Institute of Electrical and Electronics Engineers (IEEE) Transactions on Automatic Control 19:716-723.

Beier, E. III., Lehenbauer, T.W., and Sangiah, S. 1999. Clinical efficacy of fenbendazl against gastrointestinal parasites in llamas. *South American Camelids, Small Ruminant Research* In press.

Bourne, D.W.A. 1989 Boomer, a simulation and modeling program for pharmaco-kinetic and pharmacodynamics data analysis. *Computer Methods and Programs in Biomedicine* 29:191-195

Breeder and Service Directory. 1998. Oklahoma Llama Breeders Association. pp. 1-28

Cheney, J.M. and Allen, G.T. 1989. Parasitism in llamas. *Veterinary Clinics of North America: Food Animal Practice* 5(1):217-225.

Fowler, M. 1998. *Medicine and Surgery of South American Camelids.* Iowa University Press. pp 155-163.

Gibaldi, M. and Perrier, D 1975. *Pharmacokinetics*, 1st Ed. Marcel Dekker, Inc., New York. pp 37-40

Lanusse, C.E., Gascon, L.H., and Prichard, R.K. 1995. Comparative plasma disposition kinetics of albendazole, fenbendazole, oxfendazole and their metabolites in adult sheep. *Journal of Veterinary Pharmacology and Therapy* 18:196-203.

Lu, D and Mayo, F. 1993. An interactive program for pharmacokinetic modeling. *Journal of Pharmaceutical Sciences* 82:537-543.

Mayer, P.R. and Brazzell, R K. 1988. Application of statistical moment theory to pharmacokinetics. *Journal of Clinical Pharmacology.*

National Institutes of Health. 1985. *Guide for the Care and Use of Laboratory Animals.* NIH, Bethesda MD

Roberson, E.L. 1988. Antinematodal drugs. In: *Veterinary Phamacology and Therapeutics.* Booth, N.H., and McDonald, L.E. (Eds.), Iowa State University Press: Ames. pp. 877-882, 917-919, 1012-1015.

Sanyal, P.K. 1993. Plasma levels of fenbendazole metabolites in buffalo and cattlafter long term intraraminal administration. *The Veterinary Quarterly* 15(4). 157-159

Short, C.R., Barker, S.A., Hsieh, L.C., Ou, S., McDowell, T , Davis, L.E., Neff-Davis, C.A., Koritz, G., Bevill, R F. and Munsiff, I.J. 1987 Disposition of fenbendazole in the goat. *American Journal of Veterinary Research* 8(5):811-115

Short, C.R., Barker, S.A., Hsieh, L.C., Ou, S., McDowell, T., Davis, L.E., Neff-Davis, C.A., Koritz, G., Bevill, R.F. and Munsiff, I.J. 1987. Disposition of fenbendazole in cattle. *American Journal of Veterinary Research* 8(6):958-961.

CHAPTER III

CLINICAL EFFICACY PROFILES OF FENBENDAZOLE IN LLAMAS, SOUTH AMERICAN CAMELIDS

Abstract

Llamas (*Lama glama)*, South American Camelids (SAC's) are increasingly popular in the United States, as a source of fiber, livestock guard and pack animals Internal parasites have been identified as a major health problem in the livestock including llama industries. Currently there are no approved anthelmintics available for use in llamas In this study, fenbendazole was evaluated for clinical efficacy in control of gastrointestinal parasitism in llamas.

Twelve healthy young adult of both male and female llamas naturally infested with, *Nematodirus*, trichostrongyles, *Trichuris*, and *Capillaria* were divided into 2 groups. One group received a single oral dose of fenbendazole at 5 mg/kg. The second group received a comparable dose of water as a placebo. Fecal samples were obtained per rectum from each animal prior to administration of either drug or placebo treatments once a week for 4 wks. These samples were analyzed for total fecal egg burden using a modified Wisconsin sugar floatation technique. The fenbendazole treated group had a reduced total fecal egg burden of 94%, 89%, 84% and 76%, respectively, for each

week of the four week sampling period *Nematodirus,* trichostrongyles, and *Trichuris* all had significant reduction in egg production for the entire period. However, egg production by *Capillaria* was not significantly affected by the treatment. These results confirm that fenbendazole is an effective anthelmintic in the treatment of naturally occurring parasite infestations of *Nematodirus,* trichostrongyles, and *Trichuris* in llamas gastrointestinal system.

Introduction

Internal parasites are a major health problem in the livestock industry. Although there are significant numbers of "minor species" animals raised in this country for food, fiber and companion purposes, there is lack of legal (FDA-CVM approved) drugs for use in these animals. The number of Llamas (*Lama glama*) in North America is currently around 100,000 with approximately 10,000 Alpacas(*Lama pacos*), and smaller numbers of vicuña(*Vicugna vicugna*) and guanaco (*Lama guanicoe*) (personal communication). All members of the genus *Lama* have the same number of chromosomes, therefore, the physiology of these animals will be the same. In Oklahoma alone there are 45 breeding farms that are members of the Oklahoma Llama Association, with numerous others that are not registered with the association (Oklahoma Llama Breeders Association, 1998). Although little is known concerning the incidence of significant intestinal parasitic diseases and their impact on the health of South American Camelids (SAC's), there is a significant need for identifying an anthelmintic agent that is clinically effective and safe for use in these animals. Fenbendazole is a member of the benzimidazole group of anthelmintics, and has broad spectrum of activity against gastrointestinal parasites,

lungworms, and some tapeworms. This drug is currently approved for and widely used in horses, dogs, pigs and cattle (Roberson, 1988).

Fenbendazole has been studied extensively in sheep and goats, and is currently being recommended and used as an extra label anthelmintic in llamas, alpacas and other SAC's (Fowler, 1998; Cheney, 1989). However, there are no studies available concerning the efficacy and safety profiles of this drug SAC's.

Fenbendazole (Safe Guard®), at a dose of 5 mg/kg, produces a reduction in egg production of *Nematodirus, Trichuris, Strongyloides*, and *Capillaria* species, by fecal egg count (Cheney, 1989). Therefore, the major objective of this study was to characterize clinical efficacy profiles of orally administered fenbendazole in naturally infested SAC's. The results of this preliminary study will help us to determine fenbendazole's therapeutic indices

Materials and Methods

Animals

Twelve fully vaccinated, healthy, llamas of varied ages of both sexes selected from an Oklahoma farm, were used in this study. They had a significant number of parasite eggs in their feces and were not treated for parasites during the 4 months prior to this investigation.. These animals were randomly assigned to 2 groups of 6 animals each.

Animals in this study were allowed to freely graze on 50 acres of native pasture and supplemented with cracked corn and alfalfa hay once a day. They were allowed

access to 3-sided sheds, permitting the animals to get out of inclement weather. The individual animal within a group was restrained with a halter and lead rope during treatment and collection of fecal samples.

Experimental Method

Fenbendazole 10% paste (Safe Guard®, Hoechst-Roussel Agri-Vet. Sommerville, NJ) was used in the study. A single dose of 5 mg/kg, to the nearest 220 lb body weight was administered orally to individual llamas within the treatment group, using a cattle deworming gun (Hoechst-Roussel Agri-Vet, Sommerville, NJ). Individual animals in the control group received a volume of water equal to the amount of the drug as a placebo.

The llamas were monitored individually for any changes in appetite, posture, attitude, respiration, activity, temperature, urination, and defecation, every 12 hrs for 4 wks following administration of either fenbendazole or water

Collection of Fecal Samples

A minimum of 3 g of feces were collected immediately prior to administration of the drug and placebo from each animal in the study, and on every 7^{th} day thereafter for the next 4 wks. All fecal samples were obtained per rectum with a lubricated, gloved hand. These samples were stored in labeled plastic bags and refrigerated at 5°C until further analysis.

Determination of Total Fecal Egg Burden

The numbers of eggs per gram of feces were determined using a modified Wisconsin sugar flotation technique (Jordan *et al.*, 1986). Three grams of feces were placed into a clean beaker with 60 ml of water, and stirred until the feces were in a homogeneous suspension. The suspension was strained through wire gauze into a clean 150-ml glass beaker, to remove large plant fibers. An additional 60 ml of water was added to the beaker, stirred then poured over the plant material on the wire gauze, while the plant fibers were stirred with a wooden applicator The filtrate was allocated into a 15 ml centrifuge tube, which was placed into a clinical centrifuge and spun at 2000 X G for 10 min The water was decanted off of the pellet of material at the bottom of the tube and discarded A super-saturated solution of sugar was added to the centrifuge tube, and the pellet of fecal material was resuspended. Additional amount of sugar water was added to form a meniscus on the tube and covered with a 25-mm square glass cover slip. The tube was returned to the centrifuged. The cover slip was placed on a labeled clean glass microscope slide. A second glass cover slip was placed on the top of the centrifuge tube and centrifuged. This cover slip was placed next to the previous cover slip. The parasite eggs belonging to specific groups (trichostrongyles, *Trichuris*, and *Capillaria*) of gastrointestinal nematodes, were identified microscopically The total number of eggs per gram was then calculated using the following formulas;

3 gm feces /120 ml dH2O = 0.025 gm/ml

0.025 gm/ml x 15 ml = 0.375 gm/15 ml

1 ml/0.375 gm = 2.67 factor for eggs/gm of feces

Number of eggs counted on each slide x 2.67 factor = eggs/gm of feces. (Jordan *et al.*, 1986). The total egg burden with the mean ±SE of the mean was calculated for individual llamas within both the placebo and treatment groups.

Statistical Analysis of the Data,

The means ±SE of the mean of the total fecal egg burden of both placebo and fenbendazole treated groups were subjected to analysis for significance is using the SPSS program (SPSS Inc.). Due to the size of the sample number, the Mann-Whitney one tailed test, was used to determine the significance of the difference between the two groups. A one tailed Mann-Whitney was employed to determine the significance of the means within the treatment group A value of $P < 0.05$ was considered to be significant (Shott, 1990)

Results and Discussion

Oral administration of fenbendazole, at a single dose of 5 mg/kg body weight, failed to produce any adverse effects including changes in appetite, posture, attitude, respiration, activity, temperature, urination, and defecation, for the duration of the experiment These findings are consistent with the long history of safe use of fenbendazole and other benzimidazole analogues in other domestic ruminant species (Kennedy, 1975; Anderson, 1977; Gregory, 1985). Microscopic examination of the visual appearance of eggs present in the fecal material of both placebo and fenbendazole treated groups confirmed the presence of trichostrongyles, *Trichuris*, and *Capillaria* in the gastrointestinal system of the llamas. These findings are in total agreement with those of

the previous studies in llamas (Cheney, 1989; Fowler, 1998). Additionally, these groups of internal parasites have been a commonly associated with gastrointestinal parasitism in domestic ruminants of North American Continent (Kennedy, 1975; Anderson, 1977).

The total fecal egg burden of all the four groups of parasites including trichostronglyes, *Trichuris*, and *Capillaria* in both treatment and placebo groups were 54.2 ±SE and 37 ±SE, respectively, prior to either fenbendazole or placebo treatment. Oral administration of a single dose of fenbendazole at 5 mg/kg body weight, produced a reduction in total fecal egg burden of all 4 groups of gastrointestinal parasites for a period of 4 wks observed (Figure 4). Concurrently, placebo administration failed to produce any significant effects on the total fecal egg burden (Figure 4)

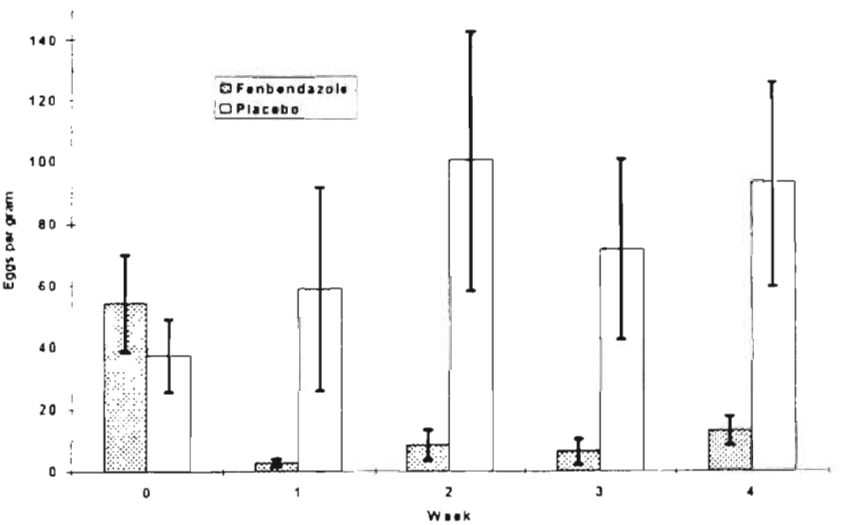


Figure 4. Mean Combined Fecal Egg Counts of Trichostrongyles in *Nematodirus*, *Trichuris*, and *Capillaria* in Llamas Over 4 Weeks Following Oral Administration of Placebo (n=6) and Fenbendazole Paste (n=6, in a Single Dose of 5mg/kg)

Based upon the analysis of fecal egg burden of individual parasites, *Trichuris* ($p > 0.004$), *Nematodirus* ($p > 0.075$) and *Tricastrongyls* ($p > 0.008$), fenbendazole has a significant effect upon reducing the numbers of eggs produced by these parasites (Figures 5 A,B,C). The number of *Capillaria* eggs was not reduced significantly by the administration of fenbendazole (Figure 5 D).

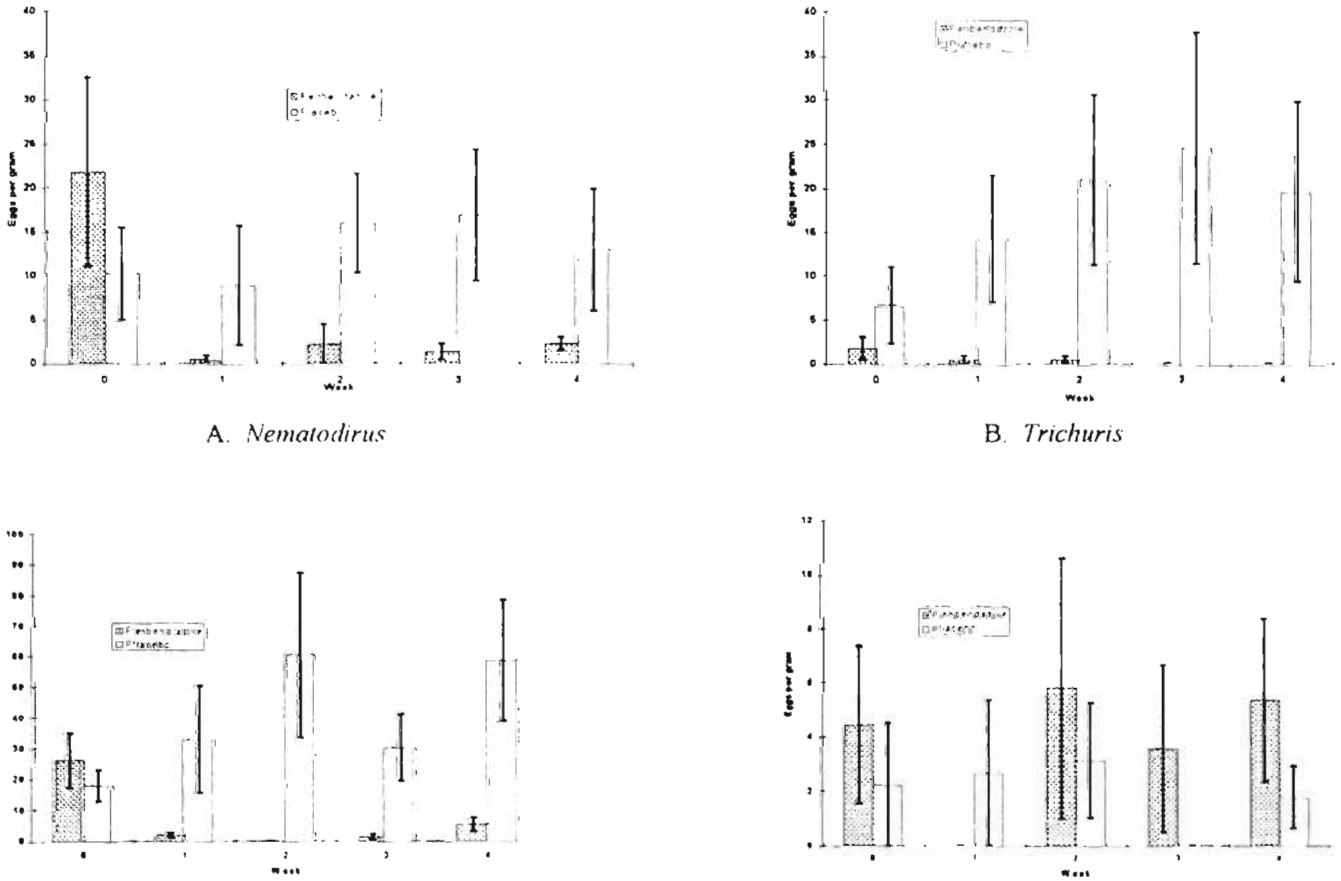
It appears that the drug did not affect this species of parasite. Perhaps this might be due to the fact that the *Capillaria* species in addition to being found in the gastrointestinal tract can also be found in the lungs, liver and kidneys Eggs produced by these parasites in the lungs are expelled into the throat in mucus, swallowed and excreted in the feces (Bowman, 1995)

The results of this study compare favorably with numerous other studies of fenbendazole on domestic ruminant species (Kennedy, 1975; Anderson, 1977).

Various methods including fecal eggs counts, larval culture, pasture larval counts, necropsy evaluation, and clinical trials, have been used to determine the clinical efficacy of an anthelmintic in domestic animals (Uhlinger, 1996; Searson, 1977, Anderson, 1977; Sutherland, 1997).

Conclusions

Using fecal egg counts, this study was the first attempt to characterize the clinical efficacy of fenbendazole in llamas. The results of this study demonstrate that fenbendazole is effective in significantly reducing the total fecal egg burden of *Trichuris* and trichostrongyles in llamas. With cash values of llamas ranging from $800.00 to $50,000.00 and alpacas even higher (personal communication), a safe



A. *Nematodirus*

B. *Trichuris*

C Trichostrongyles

D. *Capillaria*

Figure 5. Mean Fecal Egg Counts in Llamas Over 4 Weeks Following Oral Administration of Placebo (n=6) and Fenbendazole Paste (n=6, a Single Dose of 5 mg/kg). A = *Nematodirus*, B = *Trichuris*, C = Trichostrongyles, D = *Capillaria*.

efficacious anthelmintic such as fenbendazole will improve the health as well as the profitability of these animals.

More extensive studies including, larval culture and necropsy evaluation have to be condcted to further characterize fenbendazole as a safe and effective anthelmintic for llamas and other SAC's.

REFERENCES

Anderson, N. 1977. The efficacy of levamisole, thiabendazole and fenbendazole against naturally acquired infections of *Ostertagia ostertagi* in cattle. *Research in Veterinary Science* 23:298-302.

Bowman, D D 1995. *Georgis' Parasitology for Veterinarians*, 6th Ed. W.B Saunders Co., Philadelphia.

Breeder and Service Directory. 1998 "The Celebrity Show and Sale". Oklahoma Llama Breeders Association, *Llamas*, July-August, 28.

Card, C., *et al.* 1992. Report of the committee on infectious diseases of cattle, bison and llama. *Proceedings of the United States Animal Health Association* 96:61-70.

Cheney, J.M. and Allen, G.T. 1989. Parasitism in llamas. *Veterinary Clinics of North America: Food Animal Practice* 5(1):217-225

Drudge, J.H , Lyons, E.T and Tolliver, S.C. 1974 Critical and clinical test evaluations of mebendazole against internal parasites of the horse. *American Journal of Veterinary Research* 35(11) 1409-1412.

Fowler, M. 1998 *Medicine and Surgery of South American Camelids*, 2nd Ed. Iowa University Press.

Gregory, E., Foreyt, W.J. and Breeze, R. 1985. Efficacy of ivermectin and fenbendazole against lungworms. *Veterinary Medicine* 80:114-118.

Jordan, H.E., Phillips, W.A., Morrison, R.D., Doyle, J.J, and McKenzie, K. 1988. A three year study of continuous mixed grazing of cattle and sheep: Parasitism of offspring. *International Journal for Parasitology* 18(6):779-784

Kennedy, M.S and Todd, A.C 1975. Efficacy of fenbendazole against gastrointestinal parasites of sheep. *American Journal of Veterinary Research* 36(10):1465-1465.

Roberson, E.L. 1988. Antinematodal drugs. In: *Veterinary Phamacology and Therapeutics*. Booth, N.H., and McDonald, L.E. (Eds.), Iowa State University Press: Ames. pp. 877-882, 917-919, 1012-1015.

Searson, J.E. and Doughty, F.R. 1977 The efficacy of fenbendazole in treatment of naturally acquired nematode infections in cattle. *Australian Veterinary Journal* 9:456-457.

Shott, S 1990. *Statistics for Health Professionals.* W B Saunders Co, Philadelphia, pp 238-242

Sutherland, I.A., Leathwick, D.M., Brown, A.E., and Miller, C.M. 1997. Prophylactic efficacy of persistent anthelmintics against challenge with drug-resistant and susceptible *Ostertagia circumcincta. Veterinary Record* 141:120-123

Uhlinger, C.A. 1996 Parasite control programs. *Large Animal Internal Medicine*, Smith B.P., Ed. Mosby

CHAPTER IV

SUMMARY AND CONCLUSIONS

Internal parasites are a major health problem in the livestock industry Although there are significant numbers of "minor species" animals raised in this country for food, fiber and companion purposes, there is lack of legal (FDA-CVM approved) drugs for use in these animals. The number of Llamas (*Lama glama*) in North America is currently around 100,000 with approximately 10,000 Alpacas (*Lama pacos*), and smaller numbers of vicuña (*Vicugna vicugna*) and guanaco (*Lama guanicoe*) (personal communication). Little is known concerning the incidence of significant intestinal parasitic diseases and their impact on the health of South American Camelids SAC's, there is a significant need for identifying an anthelmintic agent that is clinically effective and safe for use in these animals

Fenbendazole, a member of the benzimidazole group of anthelmintics, has broad spectrum of activity against gastrointestinal parasites, lungworms, and some tapeworms. This drug is currently approved for and widely used in horses, dogs, pigs and cattle and recommended in llamas. However, there are no studies available concerning the efficacy and safety profiles of this drug SACs. In this study, oral pharmacokinetics and clinical efficacy profiles of fenbendazole were evaluated in llamas.

In the pharmacokinetic study, five healthy, young adult, male and female llamas catheterized and administered of a single oral dose of 5 mg/kg fenbendazole, 5 ml blood samples were drawn at time; 0, 15, 30, 45 min and 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 120 and 144 hrs. The plasma samples were then analyzed by HPLC for concentration of fenbendazole.

Fenbendazole enters the blood stream of llamas and reaches peak concentration of 0.28 μg/ml ± 0.129 in 10 hrs and is eliminated by 120 hrs. This peak level in plasma is comparable with those of other ruminants. The elimination rate is slower than that of sheep, cattle or goats

In the clinical efficacy study, 12 healthy, young adults of both male and female llamas naturally infested with trichostrongyles in *Nematodirus, Trichuris* and *Capillaria* were divided into 2 groups. One group received a single oral dose of fenbendazole at 5 mg/kg. The second group received a comparable dose of water as a placebo. The fenbendazole treated group had a reduced total fecal egg burden of 95%, 89%, 84% and 76%, respectively, for one through four wk sampling period. *Nematodirus*, trichostrongyles, and *Trichuris* all had significant reduction in fecal egg counts for the entire period. However, egg production by *Capillaria* was not significantly affected by the treatment. These results, confirm that, fenbendazole is an effective anthelmintic in the treatment of naturally occurring nematode infestations.

Fenbendazole appears to be a drug that is safe and effective against susceptible gastrointestinal parasites in llamas.

Vitae

Ernest Beier III

Candidate for the Degree of

Master of Science

Thesis: ORAL PHARMACOKINETIC AND CLINICAL EFFICACY PROFILES OF FENBENDAZOLE IN LLAMAS, SOUTH AMERICAN CAMELIDS

Major Field: Physiological Sciences

Biographical

Education: Graduate of Cherry Hill high School West, Cherry Hill, New Jersey in 1969; received a Bachelor of Science degree in Biology from Lynchburg College, Lynchburg, Virginia in 1973; received a Doctorate of Veterinary Medicine from the College Of Veterinary Medicine of Oklahoma State University, Stillwater Oklahoma in 1996. Completed the requirements for a Master of Science degree of Physiological Sciences in Pharmacology, from College of Veterinary Medicine of Oklahoma State University, Stillwater Oklahoma in May 1999.

Experience: Owner-operator of a Rattling Run Farms, a livestock farm in Southern New Jersey. Employed as a Laboratory Coordinator and Instructor at the School of Nursing, University of Pennsylvania, Philadelphia, Pennsylvania. Currently self employed mobile practitioner in Southern, New Jersey

Professional Memberships: American Association of Bovine Practitioners, American Association of Small Ruminate Practitioners, South Jersey Veterinary Medical Association, Delaware Veterinary Medical Association, American Veterinary Medical Association