Intestinal Crypt Lesions in an Outbreak of Albendazole Toxicity in Alpacas

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MORBIDITY & MORTALITY IN A GROUP OF ALPACA CRIAS

- **Alpaca Farm**
  - Herd of 60 alpacas
  - Central Massachusetts
  - Age range 1 month – 8 years
  - Group housed
  - No inter-group contact
HISTORY OF AFFECTED ANIMALS

• **Signalment**
  - Morbidity & mortality in 9 alpaca crias
  - Age: 1-7 months old
  - Sex:
    • 6 Females
    • 3 Males

• **Housing:**
  - 8 animals housed together in one group
  - 1 animal was housed with a separate group
  - No contact between groups
• **Background**

– These 9 animals were suspected to have cestodiasis

– Given anthelmintic treatment by the owner (only these 9 animals were treated)

– Each cria received 900mg of oral albendazole (Valbaza®) once daily for 4 consecutive days (33-100mg / kg / day)
PRESENTATION

• Two of the nine crias were found dead on the farm 1 and 3 days after receiving the last dose of albendazole
  – Presented for postmortem examination

• The other 7 animals presented to Tufts LAH within 3 days of receiving the last dose:
  – Dehydration
  – Weakness
  – Severe watery diarrhea

• Rule out infectious etiology
  – Other animals on farm at risk
INFECTIOUS DISEASES OF YOUNG CAMELIDS

• Although young camelids susceptible to similar local / generalized infections as other farm species
  – Neonatal camelids generally have:
    • Less umbilical / respiratory infections
    • More gastrointestinal infections
      – Diarrhea is an important cause of death in crias

• Gastrointestinal infections
  – Information is lacking on specific infectious agents & their pathogenicity
  – Considered susceptible to similar organisms as other farm animals
VIRUSES

• Viruses considered relatively uncommon in camelids
  – Some specific agents involved in GI disease include:

• Coronavirus
  – Little known about coronavirus in camelids
  – Increasing reports of coronavirus involvement in herd outbreaks of diarrhea
    • Beginning after a show or sale or associated with transport
      – Coronavirus is the organism isolated most consistently
    • Animals at the show affected first → herd mates develop disease 3-7d later
    • Usually self-limiting
      – Return to normal within a week

• Rotavirus
  – Has been identified as a potential pathogen
  – But little is known about rotavirus in camelids
    • Variable isolation rate in different studies
• Bovine Viral Diarrhea Virus
  
  – Rare in camelids
    • Reported in various camelid species (llamas, alpacas)
    • Research still needed to determine its full implications in camelids
    • Likely originating from BVDV-infected cattle
  
  • In alpacas:
    – Clear evidence for its existence in North American alpaca herds since 2001
    – At least 40 PI alpacas detected by early 2006
    – As in cattle, clinical signs not seen in all PI animals
  
  – Clinical signs
    • Respiratory distress
    • Diarrhea
    • Abortion
    • Congenital defects in crias
BACTERIA

• Bacteria isolated in some cases of GI disease:
  – Mostly opportunistic aerobic invaders:
    • Important contributing factors:
      – Failure of passive transfer
      – Concurrent gastrointestinal parasitic infections
  – Anaerobes not as common as in large animals:

• Clostridium perfringens
  – Cultured from some crias with diarrhea
  – Typing frequently revealed type C
  – Often in cases of enterotoxemia:
    • Concurrent infection with another pathogen
      – Enables clostridial multiplication

• Salmonella species
  – Occasional reports of septicemic salmonellosis
  – Not typically isolated from camels with diarrhea
    • Cases of enteric salmonellosis are rare in camels
PROTOZOANS

- More common cause of diarrhea in crias
  - Frequently isolated in fall / winter

- Cryptosporidium parvum and Giardia lamblia
  - Not uncommon
  - Previously considered more prevalent in older animals (2-6mo+)
  - In recent years determined important in neonates
  - Not typically isolated from healthy camelids
    - Immunocompromised
  - Cryptosporidium more common than Giardia
  - Both have zoonotic potential

- Coccidial organisms - important
  - Most common cause of diarrhea in crias
    - High intestinal loads cause diarrhea
    - Smaller loads clinically unimportant
  - Occurs in similar conditions as in ruminants
    - More commonly associated with:
      - Dirty, overcrowded buildings or pasture
      - Populations with young immunologically naïve animals
• Camelids host a specific range of coccidia:
  – Four Eimeria species infect alpacas:
    • E. macusaniensis
    • E. lamae
    • E. alpacae
    • E. punoensis

• Outbreaks of coccidiosis in alpacas
  – Usually due to E. macusaniensis & E. lamae (highly pathogenic)
    • E. lamae destroys intestinal mucosal epithelium
    • E. macusaniensis damages crypt epithelium / inhibits epithelial regeneration
    • Consequently expose intestine to secondary bacterial / viral infection
METAZOANS

- **Information scarce regarding camelid response to metazoan organisms**
  - Assumed to respond similar to ruminants
    - High intestinal loads necessary to cause diarrhea
    - Smaller loads clinically unimportant

- **Nematodes**
  - Nematodiasis occurs under similar conditions as coccidiosis (contaminated pastures etc)
  - Likely the most important are:
    - Nematodirus
    - Strongyloides
    - Trichuris
    - Capillaria
    - Ostertagia

- **Cestodes**
  - Minimal specific information documented
  - Two important species considered to be:
    - Monezia spp
    - Taenia spp
MOST SIGNIFICANT CLINICAL FINDINGS IN THE REMAINING 7 LIVE ANIMALS

HEMOGRAM:

– **Anemia:** Hct range 18-31% (Mean 16.9%)
  (Normal reference range 23-36%)

– **Leukopenia:** WBCC range 0.8 – 4.8 x 10^3 cells / µl (Mean 2.9 x 10^3 cells / µl)
  (Normal reference range: 6.1-17.7 x 10^3 cells / µl)

– **Neutropenia:** Neutrophil count range 32-3264 cells/µl (Mean 758 cells/µl)
  (Normal reference range: 3440-12730 cells/µl)

– 5 of these died despite treatment
  • Died within 3-4d post-presentation
    – Due to multiple organ failure as a complication of sepsis
POSTMORTEM EXAMINATION

• BY NOW WE HAVE 7 DECEASED ANIMALS

• Gross Postmortem Findings: Non-specific
  – Most consistent findings:
    • Reddened mucosa & serosa of the small intestine (5/7)
    • Reddened mucosa of the glandular stomach compartments (3/7)
    • Intestinal tract distended with green malodorous watery fluid +/- gas (6/7)

• Histopathologic Findings: Striking
  – In all animals, lesions were consistent in the:
    • Small intestine
    • Bone marrow
  – [Large intestinal lesions (6/7)]
  – [Glandular stomach lesions (4/7)]
Histopathology

- SMALL INTESTINE
  - Profound loss of villous architecture
  - Mucosal necrosis
  - Disrupted lamina propria
    - Crypt loss
    - Remaining crypts distended
• **Intestinal crypts**
  – Distended lumina
  – Sloughed necrotic epithelial cells
  – Regeneration of epithelial cells
    • Attenuated cells
    • Unevenly spaced
    • High N:C ratio

• **Similar lesions in the glands of the:**
  – Large intestine
  – Glandular stomach compartments
• BONE MARROW
  - Severe hypoplasia
    • Depletion of hematopoietic precursor cells
• **No cestodes** (or other metazoans)

• **Some animals did have:**
  – Cryptosporidial organisms (4/7)
  – Coccidial megaloschizonts (2/7)
ANCILLARY DIAGNOSTIC TESTING

• Intestinal contents from ileum and colon:
  – **NEGATIVE FOR VIRUSES:**
    • Rotavirus (FA) (EM)
    • Coronavirus (FA) (EM)
    • Bovine viral diarrhea virus (PCR)
  – **POSITIVE FOR AEROBIC BACTERIA**
    • Enterococcus spp.  }
    • Escherichia coli       }  *Overgrowth*
    • Acinetobacter spp.  }
  – **NEGATIVE FOR ANAEROBIC BACTERIA**
    • No evidence of salmonella or clostridial spp.
  – **NEGATIVE FOR METAZOAN PARASITES**

•  **NO EVIDENCE FOR A PRIMARY INFECTIOUS ETIOLOGY**

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WHAT CAN WE DETERMINE FROM THE MICROSCOPIC FINDINGS?

• MAJOR FINDINGS:
  – EXTENSIVE INTESTINAL MUCOSAL AND CRYPT EPITHELIAL NECROSIS WITH REGENERATION
  – SEVERE BONE MARROW HYPOPLASIA

• TARGETING RAPIDLY PROLIFERATING TISSUES
INTESTINAL CRYPT NECROSIS AND BONE MARROW HYPOPLASIA

CAUSES

• Acute radiation injury
  – Causes mitotic arrest in cells
  – Most rapidly proliferating cells targeted
    • Most mitotically active
      – Bone marrow stem cells
      – Intestinal crypt epithelial cells

• Other causes of radiomimetic lesions:
  – Viral infections: Parvoviral infection in small animals
    • Destruction of early stem / precursor cells
  – Sepsis
  – Exposure to drugs / toxins
    • ? Albendazole intoxication
ALBENDAZOLE TOXICITY

• Albendazole intoxication now strongly suspected:
  – Only albendazole-treated animals were affected
  – No exposure to radiation
  – No overt evidence of a viral or other infectious etiology
  – Sepsis was considered a secondary event
ALBENDAZOLE

• A member of the benzimidazole anthelmintic class
  – The most recently introduced benzimidazole

• Exceptional broad-spectrum activity
  – One of the main groups used clinically
  – Drug of choice for most nematode infections
  – Orally administered
ALBENDAZOLE DOSING

• Oral dosing regime for effective treatment varies with species
  – Repeated administration (humans, pigs, dogs, cats)
  – Single dose (ruminants, horses)

• Suspension widely used & approved for cattle & sheep
  – Cattle: 10mg / kg (one-time dose)
  – Sheep: 7.5mg / kg (one-time dose)

• Llamas and alpacas:
  – Commonly used in camelids
  – Without reported adverse affects
  – No approved dose
    • Anecdotal dose range 10-15mg / kg (one time dose)
    • These crias received 33-100mg / kg / day for 4 days
MECHANISM OF ACTION OF ALBENDAZOLE

• Mode of action of anthelmintics
  – Most affect parasite neuromuscular system
  – Albendazole is different: exerts its action on the cytoskeleton

• Cytoskeleton “101”
  – A dynamic structural and functional framework in the cell
    • Composed of 3 types of filaments
      – Microtubules
        » Targeted by albendazole
      – Microfilaments
      – Intermediate
Microtubules

- **Important functions**
  - **Scaffold**
    - Maintain shape & framework of cells
  - **Cell transport**
    - Intracellular movement of organelles
  - **Cell division**
    - Form the mitotic spindle
    - Originate from centrosomes
    - Shorten & separate diploid chromosomes in mitosis

www.beyondbooks.com
Microtubules

• **Dynamic structures**
  – Continually assemble & disassemble

• **Composed of tubulin**
  – A dimer protein
  – Consists of two monomers
    • \( \alpha, \beta \) tubulin
    • Dimerize & polymerize during microtubule assembly \( \rightarrow \) ultimately disassemble

• **Albendazole**
  – **Inhibits microtubule formation**
    • Binds specifically to \( \beta \)-tubulin
  – Inhibits mitosis
Mechanism Of Action

- Albendazole preferentially affects parasite tubulin
  - Selectively damages microtubules in the nematode intestinal cell cytoplasm (not the host cells)
  - Inhibits tubulin polymerization → uncouples oxidative phosphorylation & blocks microtubule-dependent uptake of glucose by the nematode intestine:
    - Disrupts absorptive & secretory functions essential to parasite survival
      - Glucose uptake is reduced
      - Diminished energy supply immobilizes & kills the parasite
Albendazole Toxicity Study Data

- Data from animal studies involving repeated dosing of albendazole
  - Level causing no effect (NOEL) (rat, rabbit, dog)
    - 5-7mg/kg/day (2 year study)
  - Mouse, rat, and dog (30-40mg/kg/day for 4-90 days)
    - Reduced weight gain, anemia, mild leukopenia, hypercholesterolemia
  - Wistar rat (60-120 mg/kg/day for up to 90 days)
    - BM hypoplasia with pancytopenia

- These crias received 33-100mg / kg / day for 4 days
Albendazole Toxicity in Passerine Birds  
(Zoological Society of San Diego)

- **PIGEONS AND DOVES**
  - Suspected toxicity problems with albendazole treatment
  - Retrospective study to identify clinical signs or toxic lesions associated with albendazole treatment
  - Evaluated hospital / PM records for 410 birds

  - Only included birds hospitalized for non-medical reasons
    - Quarantine / Preshipment examination / Routine parasite screening
  - Albendazole (n=36) treated group: (50–100 mg/kg PO q24h for 3–10 days)
  - Fenbendazole (n=84) treated group / Control group (n=290)

J Avian Med Surg 16(3) 2002
Albendazole Toxicity in Passerine Birds
(Zoological Society of San Diego)

- Findings in the albendazole-treated group (n=36)
  - **Marked leukopenia:** <1000 WBC / µl: 100% (P=0.013) (Control 0%)
    
    *(Leukopenia in birds - identified as total WBCC <3000 / µl)*

  - **Weight loss:** Mean 13.3% bodyweight: 100% (P=0.004) (Control animals gained weight)

  - **↑ Morbidity / mortality:** 66.7% survival (P<0.001) (Control 90%)

  - **Cause of death:** Peracute bacterial / fungal infection Consistent with immunosuppression

J Avian Med Surg 16(3) 2002
Albendazole Toxicity in Passerine Birds
(Zoological Society of San Diego)

• **NECROPSY FINDINGS**
  – **Histopathology**
    • Small intestinal crypt epithelial necrosis: 58.3% (P<0.001) (Control 0%)
    • Bone marrow hypoplasia: 83.3% (P<0.001) (Control 0%)
  
  – **Conclusions:**
    • Lesions consistent with albendazole intoxication
    • Passerine birds are susceptible to albendazole toxicity
Albendazole Toxicity in Humans

- Albendazole in humans
  - Enviable low toxicity record
  - Most commonly used as a short term treatment
    - Passive reporting of side effects initially minimal
      - Rare, idiosyncratic reactions reported
  - Treatment of echinococcosis / cysticercosis
    - Long term treatment (weeks to months)
      - 7-15mg/kg/day
    - Increasingly common since 1980’s
    - Toxicity data slowly emerging
  - Repeated dosing produces:
    - Bone marrow hypoplasia
      - Severe neutropenia
      - Aplastic anemia
      - Thrombocytopenia
      - Pancytopenia

Autopsy bone marrow specimen
(Magnification x70)

Bone marrow hypoplasia:
68 year old male treated for
3 weeks with albendazole @
400mg BID for a pulmonary
echinococcal cyst

Am J Trop Med Hyg 72(3) 2005
CASE SERIES: OUTCOME

• 2/9 original animals remained alive
  – Crias # 6 and # 5
    • Hospitalized for 17 and 24 days respectively
      – Given supportive care:
        » Broad-spectrum antimicrobial treatment
        » Intravenous fluid therapy

• Progressively neutropenic over 3 days
  – **Cria # 5**: 3264 → 8 cells / µl
  – **Cria # 6**: 216 → 0 cells / µl

(Normal reference range 3440-12730 cells/µl)
• Crias #5 & #6 were treated with filgrastim (Neupogen®)
  – 5µg/kg S/C q24h

• Filgrastim
  – Recombinant human granulocyte colony-stimulating factor (rG-CSF)
  – Hematopoietic growth factor
    • Controls proliferation of committed progenitor cells
      – Regulates production and function of neutrophils
        » Stimulates their release from BM storage pools
        » Reduces their maturation times
Neutrophil counts

Within 7 days, neutrophil counts normalized
Case Series: Outcome

• **Cria #6:**
  
  – **Persistent pyrexia**
    • Failed to resolve despite broad-spectrum antimicrobial/antifungal agents
    • Negative blood-cultures
    • No abnormalities on thoracic radiographs or abdominal ultrasound
  
  – **Developed severe thrombocytopenia 10d post-admission**
    • 5000 platelets / µl (Normal reference range 100-400 x10³ cells / µl)
    • Treated with a whole blood transfusion
  
  – **Diagnosed with erythrocyte parasitization by *Mycoplasma haemolama***
    • Diagnosed 21 days post-admission
    • Given oxytetracycline (10mg/kg SC, q48h)
      – Pyrexia resolved within 36 hours
Case Series: Outcome

- Both crias #5 and #6 were discharged
- Did well on the farm for a period of time
  - Gained weight
- Cria #6 died suddenly 3 months after discharge:
  - No direct association made with earlier albendazole toxicity episode
- Cria #5 is still thriving on the farm
- Final veterinary bill >$30,000
CONCLUSION

• **Cause of morbidity and mortality in these crias was attributed to albendazole toxicity:**
  – Only the albendazole-treated group were affected
  – Lesions identical to cases reported in other species

• **Repeated daily dosing inevitably contributed to the toxicity**
  – 33-100mg / kg / day for 4 days

• **Albendazole may have a narrow therapeutic index in alpacas**
  – ? Possible increased sensitivity of alpacas to albendazole
    • Similar to that seen in passerine birds

• **Recommend a one time maximum dose**
  – 10-15mg / kg
Thank you!