

Journal of Veterinary Medicine and Animal Health

Volume 5 Number 2 February 2013



*Academic
Journals*

ABOUT JVMAH

The **Journal of Veterinary Medicine and Animal Health (JVMAH)** is published monthly (one volume per year) by Academic Journals.

The **Journal of Veterinary Medicine and Animal Health (JVMAH)** is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject like the application of medical, surgical, public health, dental, diagnostic and therapeutic principles to non-human animals.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JVMAH are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jvmah@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Veterinary Medicine and Animal Health (JVMAH) will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Fuqiang Li PhD

Division of Cardiology
Department of Medicine
Cedars-Sinai Medical Center
8700 Beverly Blvd
CA 90048
USA

Dr. Lachhman Das Singla

Department of Veterinary Parasitology
College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences University
Ludhiana-141004
Punjab
India

Dr. Viktor Jurkovich

Szent István University,
Faculty of Veterinary Science,
István utca 2. H-1078 Budapest
Hungary

Dr. Sathurkulasingam Reuben Shanthikumar

606 Alvarado Avenue
Apt # 64, Davis, CA 95616
USA

Dr. Adeolu Alex Adedapo

Department of Veterinary Physiology
Biochemistry and Pharmacology
University of Ibadan
Nigeria

Prof. Anca Mihaly Cozmuta

Faculty of Sciences
North University of Baia Mare
Romania, Victoriei Str. 76 A, Baia Mare
Romania

Dr. Ramasamy Harikrishnan

Faculty of Marine Science
College of Ocean Sciences
Jeju National University
Jeju city
Jeju 690 756
South Korea

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types

Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JPP to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The **Title** should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The **Abstract** should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The **Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:

Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included

in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Ansell J, Hirsh J, Poller L (2004). The pharmacology and management of the vitamin K antagonists: the Seventh ACCP Conference on Antithrombotic and Thrombolytic. Therapy. 126:204-233

Ansell JE, Buttaro ML, Thomas VO (1997). Consensus guidelines for coordinated outpatient oral anti coagulation therapy management. Ann. Pharmacother. 31:604-615

Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

Furmaga EM (1993). Pharmacist management of a hyperlipidemia clinic. Am. J. Hosp. Pharm. 50: 91-95

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences:

(1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a \$550 handling fee. Publication of an article in the Journal of Veterinary Medicine and Animal Health (JVMAH) is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Copyright: © 2013, Academic Journals.

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the JVMAH, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.

ARTICLES

Research Articles

- Expedited management of ulcer, colic and diarrhea in 209 horses: An open-labeled observational study of a potency-enhanced sucralfate-like elm phyto-saccharide** 40
Ricky W. McCullough
- Prevalence of bovine coccidia in Kombolcha district of South Wollo, Ethiopia** 45
Alula Alemayehu, Mohammed Nuru, Timketa Belina
- Gross pathological changes in the reproductive tracts of cows slaughtered at two abattoirs in Southern Ethiopia** 50
Berhanu Mekibib, Techan Desta, Dawit Tesfaye
- Occurrences and financial significance of bovine cystic echinococcosis in Southern Wollo, Northeastern Ethiopia** 56
Alemu Bizuwork, Nigatu Kebede, Tariku Tibat, Getachew Tilahun, Tesfu Kassa
- Molecular detection of canine parvovirus in Jos, Nigeria** 59
S. C. Chollom, E. J. Fyaktu, A. E. J. Okwori, G. O. A. Agada, G. Hashimu, R. Y. Akele, E. I. Voumangai, T. Dashe, D. Z. Egah

Full Length Research Paper

Expedited management of ulcer, colic and diarrhea in 209 horses: An open-labeled observational study of a potency-enhanced sucralfate-like elm phyto-saccharide

Ricky W. McCullough

Department of Medicine and Emergency Medicine, Providence Veterans Administration Hospital, Brown University Teaching Hospital, 830 Chaulkstone Avenue, Providence Rhode Island, United States. E-mail: glencopelph@aol.com.
Tel: 401-397-6203. Fax: 401-397-8559.

Accepted 10 December, 2012

A potency-enhanced polyanionic phyto-saccharide of elm mucilage (PEPPS) was prescribed in 209 horses in an open-labeled field trial. Clients provided informed consent to 32 equine veterinarians to prescribe PEPPS for low grade colic and diarrhea unresponsive to deworming. Most of the horses (n = 175/209) were presumed to have ulcers clinically, while 23/209 were confirmed by gastroscopy to have ulcers and 11/209 horses had colic related to diarrhea. A 4-day/7-dose response rate, determined by veterinarians' consensus, provided the threshold for a significant clinical outcome, and data was collected through phone interviews over period of 3.5 years. 166/198 horses with ulcer associated colic and 10/11 horses with diarrhea-associated colic responded to PEPPS within 4 days or 7 doses. Using PEPPS alone was associated with positive outcomes in 81% (29/36) of horses. PEPPS added to failed regimens of omeprazole, ranitidine and antacids was associated with positive outcomes in 85% (137/162) of horses. Accelerated healing of ulcers occurred in 8 horses within 14 to 20 days using PEPPS alone and in 15 horses using PEPPS added to failed treatment regimens of omeprazole, ranitidine and antacid. PEPPS appears useful for managing equine ulcer, diarrhea and colic. As an electronegative polyanionic saccharide with substantial muco-adherence, PEPPS was compared to sucralfate. However, a randomized blinded placebo controlled trial is needed to quantify true clinical efficacy.

Key words: Equine, ulcer, diarrhea, colic, elm, sucralfate.

INTRODUCTION

Timely management of disruptive gastrointestinal (GI) symptoms poses a challenge to veterinary physicians (Mair et al., 2002; Tams, 2003). Inappetence, ulcer colic and diarrhea in horses can lead to more severe problems. Therefore, restoration of normal GI function is paramount and management should be expedited. The problem of disruptive equine GI symptoms is fairly

significant. Fifty to 92% of the 9.2 million horses in the US (American Horse Council, 2005) have gastric ulcers, depending on their breed and type of containment or athletic activity (Hammond et al., 1996; Mairs et al., 2002; Murray et al., 1996; USDA APHIS, 2001a; Vatistas et al., 1994). In "gastroscopy clinics" conducted over 12 months in 25 states in the US, 60% of 658 horses were found to have ulcerations (Knudson, 2009). Symptomatic ulcers usually present as low grade colic with food avoidance or inappetence. Inappetence is a principal sign to horse owners of colic and often serves as an early warning for the presence of ulcers. When surveyed, horse owners in Minnesota ranked ulcer colic 5th out of 25 top equine healthcares concerns (Martinson et al., 2006).

Abbreviations: GI, Gastrointestinal; HPS, high potency sucralfate; PEPPS, potency-enhanced polyanionic phyto-saccharide; USDA, United States Department of Agriculture; FDA, food and drug administration.

The USDA National Economic Cost Report on colic (2001b) reporting no geographic variation in the incidence of colic in horses, imply that this sentiment of concern for colic could be generalized to all horse owners. General prevalence positions ulcer colic as a significant disruptive GI symptom in horses and one that require effective management (Hillyer et al., 2001; Kaneene et al., 1997; Tinker et al., 1997; Traub-Dargatz et al., 2001).

Evidence-based treatment of equine diarrhea and non-surgical colic is limited. However, some published reports (Galvin et al., 2004) suggest that colic can be treated with pain medications such as xylazine (0.5 mg/kg) or butorphanol (0.01 mg/kg). The use of an anti-spasmodic is a reasonable option as well. The initial management of diarrhea entails reducing the mechanical load of the colon chiefly by elimination of large volume fiber sources. Low bulk diet is complimented by the use of psyllium, corn oil and bismuth/salicylate suspension by nasogastric tube which is a current management of equine mucosal erosions, ulcerations and resulting colic and inappetence centers on therapeutic control of acidity (Andrews, 2005; Hammond et al., 1996; Hillyer et al., 2001; Knudson, 2009; Merial, 1999). Acidity is either neutralized by antacids (Andrews, 2005), reduced by histamine-2 blockers (for example, ranitidine, cimetidine, famotidine) (Andrews, 2005) or inhibited by proton pump inhibitors (for example, omeprazole) (Anderson, 2005; Knudson, 2009; Merial, 1999). Neither of these therapeutic agents provides direct relief of colic or direct healing of the mucosa. Instead, colic relief and ulcer healing are secondary consequences of lowering acidity. There are genetically controlled mucosa-specific mechanisms unrelated to acidity that are primarily responsible for direct healing (Tarnawski et al., 1998).

Proof of the presence of such mechanisms is found in FDA trial data on omeprazole (Merial, 1999). There was significant rate of healing observed in horses randomized to sham treatment. While acidity significantly hampered the rate of healing, nevertheless, complete healing occurred in 40% of untreated horses. This of course, was not a "mind over matter" placebo effect, but rather a genetically orchestrated phenomenon that is entirely independent of gastric pH (Jones et al., 1999; Konturek et al., 1991; Pai and Tarnawski, 1998; Skov, 1988; Tarnawski et al., 1998). Sucralfate is a polyanionic saccharide with cyto-protective properties. By composition, it is octa-sulfonated disaccharide containing glucose and fructose. The electro-negativity of its eight sulfate groups imparts charge density that favors muco-adherence to the GI lining (Dobrozsi et al., 1999; Jubeh et al., 2004; Tarnawski et al., 1987). Muco-adherent binding of sucralfate to GI lining is indifferent to gastric acidity (Steiner et al., 1982) with its strength of binding unaffected by pH (Danesh et al., 1988). Additionally, by virtue of its electro-density, sucralfate binds enteric growth factors (Jones et al., 2004; Konturek et al., 1991, 1995; Szabo, 1991; Szabo et

al., 1994) and thereby facilitates immediate mucosal regeneration (Tarnawski et al., 1986). The same electro-negative density that enables it to modulate calcium channels of the gastric mucosa (Slomiany et al., 1992) may also be responsible for repolarization of voltage-gated nociceptors innervated by the vagus nerve which are known to be responsible for pain sensation from acid (Holzer, 2001, 2004), nausea and vomiting (Beyak and Grundy, 2005). The latter quality is significant and may account for the fact that in small animals, when administered in multiples of the standard 14 mg/kg per dose (that is 25 to 100 mg/kg/dose), sucralfate stops vomiting and diarrhea in 2 to 4 doses (Steiner, 1990). When suspended in a solution of cations and multi-dentate chelators, the electronegative density is multiplied through self-aggregation leading to a multifold augmentation of surface concentration of sucralfate and thereby augmentation of its potency (McCullough, 2010), since the entire therapeutic effect of sucralfate is defined by the quantitative extent of its physical contact with the GI lining.

Besides positive anecdotal reports (Translational Medicine Research, 2002), there is no published data on ulcer response to potency-enhanced sucralfate. Slippery elm mucilage is a polyanionic phytosaccharide version of sucralfate. It also contains branched substituents with a high electronegative density, (galacturonic acid). When placed in a solution of cations and multi-dentate chelators, its muco-adherence is potentiated several fold (McCullough, 2010). As such, it is referred to as a potency-enhanced polyanionic phyto-saccharide or PEPPS. The clinical effects of PEPPS in humans and small animals (anti-emetic, anti-diarrhea, anti-ulcer) are indistinguishable from that of potency-enhanced sucralfate. Therefore, it is assumed that PEPPS has physiochemical characteristics similar to sucralfate, being muco-adherent, preferentially engaging mucosa-adherent growth factors and capable of repolarization of voltage-gated nociceptors related to acid pain and nausea. Though PEPPS has been prescribed by veterinarians in the US since 2003 for equine ulcer colic and diarrhea, its use on a large scale has never been formally reported. This report presents observational data in a proof of principle study which may be useful (Dreyer et al., 2010) in prescribing PEPPS in the setting of supervised treatments in an equine practice. Of course, any therapeutic intervention positively associated with the management of inappetence, ulcer colic and diarrhea would be a constructive development.

MATERIALS AND METHODS

Potency enhanced polyanionic phyto-saccharide

Elm mucilage is a polyanionic phyto-saccharide (Upton et al., 2011). Unlike sucralfate, PEPPS contains no aluminum or sulfate. It is chiefly a high molecular weight mucilage (> 200,000 Daltons), comprising of galactose-rhamnose disaccharides. Potency-enhanced elm phyto-saccharide is prepared by suspending elm

mucilage in an anion-cation solution similar to that used to formulate high potency sucralfate (HPS) (McCullough, 1995, 2010, 2012). The resultant potency-enhanced phyto-saccharide (PEPPS) is muco-specific and capable of attaining augmented surface concentration of slippery elm. With sucralfate, potency enhancement ranges from 7 to 23 fold 3 h post-administration, having a lower fold increase on normal GI lining and higher fold increase on inflamed or injured mucosa. The exact post-administration surface concentration of PEPPS is unknown. However with PEPPS, the concentration of elm USP administered is less than 2% of slippery elm dose recommended by equine veterinarians (Veterinary Desk Reference, 2011; Ward, 2010). The formulary strength of Elm USP for horses is 1.9%. Administration of PEPPS is in accordance to weight. On average, horses weighing less than 500 lbs received daily doses upwards of 270 mg, those between 500 to 900 lbs received 380 mg and over 900 lbs received 540 mg. PEPPS was administered separate from other medications.

Dosing administration

Participating veterinarians prescribed PEPPS in accordance to weight-dose chart in label instructions. Horses weighing less than 500 lbs (227.3 kg) received 15 ml, between 500 to 900 lbs (227.3 to 409.1 kg) received 20 ml, and over 900 lbs (409.1 kg) received 30 ml. PEPPS was given by drench into cheek pouch twice daily, morning and evening. PEPPS is not absorbed systemically and act as an internal topical coating the GI lining.

Study design and controls

This study was an open labeled non-blinded observational trial. Observational trials are fundamentally distinct from a randomized controlled clinical trial, the least of which there is no control group. Merit is assessed on the basis of outcomes resulting from the introduction of an intervention into a pre-existing clinical setting (Hannan, 2008). Information was collected regarding; the age of horses, the nature of their GI symptoms (the presence of ulcer-suspected colic and diarrhea in horses), and type of treatment regimens prescribed by the practitioner at time of adding PEPPS. The length of illness is not reported. As an observational study, treatment intervention was not randomized. By design, differences in outcomes are observed without regard to similarities or dissimilarities of patient characteristics prior to treatment. In fact, in this type of study, treatment decisions were made by veterinarians prior to use of PEPPS; the selection of PEPPS being made by the veterinarian due to concern that pre-PEPPS treatments were ineffectual.

In this trial the question addressed is not one of the efficacies of PEPPS. Instead, the question addressed is one of the relative merits of PEPPS as a competing treatment or intervention. Outcome of merit is relative to the expectation of the participating veterinarians. As discussed below, a clinical response of 4 days or 7 doses merited note to the veterinarians involved. This study reports the percentage of horses with ulcer related colic and diarrhea related colic who responded to PEPPS while on failing therapies.

Comparative control

As an observational study, there were no control groups (Hannan, 2008). To provide a comparative “control” experience, each veterinarian was asked to reflect on their respective experience and select from a choice of a clinical response times which they would

deem to deviate significantly from the expectations of their clinical experience. Most of the small animal veterinarians (80%) felt that a clinical response of 4 days or 7 doses would mark a significant departure from their clinical expectations, and this was based on their experience managing ulcer- and diarrhea associated colic in horses. This consensus of significant departure from expected time of clinical response was used to benchmark the primary outcome and a meaningful response. In essence, expectations of past clinical experience (replete with interventions requiring more time to work) served as a “comparative control” albeit a subjective one.

Consent

All animals were privately owned and owners’ consent was obtained by veterinarians.

Veterinarians participating in the study

Veterinarians placing orders for commercially available PEPPS were recruited to participate in this open-labeled trial. None had professional experience less than 5 years in practice. Veterinarians were familiar with using 2002 original formulation of equine Gastrafate® which contained 10% high potency sucralfate as the active ingredient. Following successful preliminary testing (Translational Medicine Research Center, 2002) high potency sucralfate was replaced in January, 2003 with calcium chelated elm mucilage in the form of PEPPS. Veterinarians were recruited from June, 2003 through December, 2006. All veterinarians prescribing PEPPS were engaged exclusively in primary care of equine animals within valid veterinarian-client-patient relationships. The combined years of practice for equine practitioners who completed the trial were 160. Each was experienced in the standards of care in treating ulcer- and diarrhea associated colic in horses. Out of 46 equine veterinarians, 32 practicing in 20 states of the US completed the study; the remainder lost to follow due to their inability to complete protocol. Veterinarians received no honorarium for their participation.

Sequential participation

Participation in the study was sequential, determined solely by the order of spontaneous requests for product made by veterinarians responding to notification of product’s availability. The veterinarians were self-selected. Information prompting orders pertain to the usefulness of PEPPS in the management of ulcers- and diarrhea associated colic in horses.

Inclusion/exclusion criterion for horses

Included in the observational trial were horses with low grade colic, inappetence or diarrhea for more than 5 days. These horses had failed adequate deworming therapies by owners. Horses were with and without a history of ulcers confirmed by endoscopy. Endoscopy was not required for inclusion. However, in accordance to customary practice of 8 veterinarians, 23 horses underwent endoscopy before and after treatment.

Test population

Horses of all age groups, breeds and ownership purposes were included. The size of the test population was 209 horses within a multi-practice-based study. There were 20 foals under 6 months, 38

between 6 months to a year, 33 between 1 to 2 years and 118 over age 2. The test population was geographically diverse involving input from 20 out of the top 25 states with highest horse ownership and equine population. As was the custom for 8 of the 32 equine practitioners, 23 horses with ulcer-related colic underwent endoscopy prior to and following treatment with PEPPS.

Conditions managed by equine practitioners

Conditions to be managed in the trial included low grade (non-surgical) colic, ulcer-suspected colic and diarrhea. Low grade colic was heralded by poor appetite or refusal to eat – symptoms that failed to respond to owners' use of dewormers or adjustments in feed. Ulcer-suspected colic was defined clinically as inappetence (poor feeding/drinking), poor attitude, dull coat and teeth grinding in foals. Diarrhea-associated colic was defined as symptoms identical to ulcer-suspected colic accompanied by loose stools for more than 5 days despite adequate de-wormer therapy by owners. Endoscopy in 23 horses was positive for gastric ulcers. Excluded were horses whose symptoms required surgical intervention.

Conduct of observational field tests

The study was conducted from June, 2003 through December, 2006. Participating veterinarians prescribed PEPPS in accordance to weight-dose chart in label instructions. The dose administration was three times daily for the first day then twice daily. Phone interviews were conducted with veterinarian staff to collect results of adding PEPPS to existing treatment regimens. Results were tabulated as either a positive or negative outcome.

Pre-existing treatment regimens used in study horses

Pre-existing treatment regimens for horses were fairly uniform and centered around four regimens. These were (a) omeprazole alone, (b) omeprazole + antacid, (c) histamine-2 blocker (ranitidine or cimetidine) + antacid or (d) histamine-2 blocker (ranitidine/cimetidine) + antacid + bismuth suspension for diarrhea-associated colic. The antacid used was aluminum hydroxide/ magnesium hydroxide combination. The length of time horses had been on these regimens was not assessed, therefore unknown. When clinical results from pre-existing regimens were deemed suboptimal, participating veterinarians added the polyanionic saccharide, PEPPS.

Horses treated with PEPPS alone

This open-labeled trial was observational with no control groups of untreated horses. However, a small number of horses, 36 of 209 formed a PEPPS only treatment group. Except for a few veterinarians treating the 36 PEPPS only group, previous experience of 80% veterinarians offered no expectation of significant response to a PEPPS intervention within 4 days or 7 doses.

Primary outcome measure in horses

There were two single symptom-related primary outcome measures for this trial. Cessation of ulcer colic and diarrhea were the two single symptom outcomes, respectively. Veterinarian-defined positive responses in horses with ulcer colic and diarrhea would be the restoration normal feed habits and the return of formed stools within 4 days or 7 doses of PEPPS, respectively. This veterinarian-

defined response to therapy previously discussed represented a meaningful clinical difference for the management of ulcer colic and diarrhea in horses.

Hypothesis

The hypothesis is that majority of animals with serious and disruptive GI symptoms (of non-surgical etiology) when given PEPPS will experience resolution of symptoms within a timeframe (or dose administration), significant and relevant to the collective historical experience of practicing veterinarians who manage such symptoms routinely. This was a timeframe of 4 days (or in 7 doses).

Analysis

The trial used a per protocol analysis of the data. Both chi-square and two-sample t-test were used to compare percent response to treatment among horses at confidence level of 95 and 99% for confidence intervals.

RESULTS

General

Horses with ulcer-related colic and diarrhea-related colic

All horses eventually responded to PEPPS with variability in the clinical response times extending beyond 4 days. However, Tables 1 and 2 showed that 85% [CI 5.47 (CL 95%)] of 198 horses with ulcer-associated colic and 91% [CI 16.91(CL 95%)] of 11 horses with diarrhea-associated colic responded to PEPPS within 4 days or 7 doses. The percent response using PEPPS alone to manage ulcer-associated colic was 81% [CI 12.9/CL 95%, n = 36]. When PEPPS was added to existing treatment regimens, the percent response was high. Percent response was 83% [CI 10.11/CL 95%] when PEPPS was added to omeprazole (n = 53), 81% (CI 12.64/CL 95%) with PEPPS added to omeprazole + antacids (n = 37), 88% (CI 7.26/CL 95%) with PEPPS added to ranitidine/cimetidine + antacids (n = 72), and 91% (CI 16.91/CL 95%) with PEPPS added to ranitidine/ cimetidine + antacids + bismuth (n = 11).

PEPPS alone compared to PEPPS augmented treatments

Table 3 shows the results of a two-sample t-test used to compare the percent response using PEPPS alone to the percent response using PEPPS augmented treatments. In each comparison, PEPPS alone versus (PPI + PEPPS), PEPPS alone versus (PPI + AA + PEPPS), PEPPS alone versus (H2B + AA + PEPPS), or PEPPS alone versus (H2B + AA + Bismuth + PEPPS), the t-statistics was not significant at the 0.05 critical alpha levels. There was no statistically significant difference between treatment with PEPPS alone and treatment with PEPPS augmented

Table 1. Treatment response to PEPPS† stratified by GI symptoms, treatment regimens and age of horse.

Treatment regimen	< 6 months (%)	6-12 months (%)	1-2 years (%)	2-8 years (%)	> 8 years (%)	Symptom/disorder treated	Treatment related totals (%)
H2B+AA+Bismuth+PEPPS	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/3 (67)	Colic-diarrhea	10/11 (91)
H2B+AA+PEPPS	2/2 (100)	11/13 (85)	9/11 (82)	25/27 (93)	17/19 (89)	Ulcer colic	64/72 (88.9)
PPI+AA+PEPPS	3/4 (75)	6/7 (86)	6/7 (86)	10/13 (77)	5/6 (83)	Ulcer colic	30/37 (81.0)
PPI alone+PEPPS	4/4 (100)	10/13 (77)	6/8 (75)	18/21 (86)	6/7 (86)	Ulcer colic	44/53 (83.0)
PEPPS† alone	7/8 (88)	3/3 (100)	4/5 (80)	10/13 (77)	5/7 (71)	Ulcer colic	29/36 (80.6)
Age Related Response	18/20 (90)	32/38 (84)	27/33 (82)	68/76 (90)	35/42 (83)	180/209 (86)	177/209 (84.7)

PEPPS, potency-enhanced polyanionic phyto-saccharide; PPI, proton pump inhibitor (omeprazole); AA, antacids; H2B, histamine-2 blocker (ranitidine or cimetidine); bismuth, bismuth preparations.

treatments. No additional clinical response was obtained over PEPPS alone by the continuance of pre-existing therapies (omeprazole, ranitidine, anti-acids or bismuth).

Endoscopy positive ulcers in horses

Of the 198 horses with ulcer-associated colic, 23 horses were endoscoped by 8 veterinarians prior to and between 14 to 20 days, following PEPPS treatment. Eight of these horses were on PEPPS alone, while the other 15 were on PEPPS augmented therapies. Table 4 shows that while horses in each treatment group had ulcers, all ulcerations were confirmed healed by second endoscopy performed between 14 to 20 days on treatment. With FDA, approved omeprazole require 28 days for a statistically significant healing effect, this observation of PEPPS healing between 14 to 20 days represented accelerated healing.

DISCUSSION

General impression

Traditionally, the treatment of ulcer colic in horse

generally centers on acid reduction (Andrews, 2005; Hammond et al., 1996) for which there are several therapeutic options. Clinical practice to treat equine diarrhea involves the use of psyllium hydrocolloids and bismuth/salicylate combination (Galvin et al., 2004). The empiric use of anti-spasmodics, analgesics and anxiolytics for colic is reasonable as well. However, simultaneous management of ulceration, diarrhea and colic by a single therapeutic agent would be preferred. Potency-enhanced polyanionic phyto-saccharide, PEPPS was prescribed to 209 horses in the private practices of 32 equine practitioners in the US. With the exception of a few cases, the equine practitioners introduced PEPPS into active treatment regimens, deemed to be failing. The study was conducted over a 3.5 year period. The data showed a positive association between the use of PEPPS and the rapid resolution of ulcers, ulcer-associated colic and diarrhea in horse whose symptoms had failed to respond to pre-existing therapies. The hypothesis that the administration of PEPPS is associated with the rapid resolution of ulcer colic, diarrhea and diarrhea associated colic in most horses was supported by the data reported here. In majority of horses on failing therapies, those symptoms resolved within 4 days (or in

7 doses) of receiving PEPPS, a timeframe significantly less than that anticipated from the private practice experiences of veterinarians participating in the trial. Additionally, all horses with ulcerations diagnosed by gastroscopy healed within 14 to 20 days using either PEPPS alone or PEPPS augmented by continuance of existing treatments. In other words, the use of PEPPS in horses with known gastric ulcers was associated with the resolution of ulcer within 14 to 20 days, which is sooner than 28 day therapy required for omeprazole (Merial, 1999) to achieve statistically significant healing effect. A more fair comparison of PEPPS to omeprazole requires evaluation of both under similar clinical restrictions. Be that as it may, 14 to 20 days for ulcer healing is far less than 28 days known to be required for omeprazole.

Limitations of this observational study

Observational study of this nature does have distinct limitations (Hannan, 2008). The absence of conventional control groups precludes any statements regarding efficacy. An observational study shows association without commentary to cause. Instead, what is known from this study is

Table 2. Percentage response to treatment in horses with CI (95%).

Parameter (Treatment regimen)	Horses with ulcer colic	
	Responsive	CI (95%)
PEPPS Alone	81% (n=36)	12.9
PPI + PEPPS	83% (n=53)	10.11
PPI + AA + PEPPS	81% (n=37)	12.64
H2B + AA + PEPPS	88% (n=72)	7.26
Ulcer colic regimens 2,3,4 collectively	85% (n=162)	5.47
Horses with diarrhea and colic		
H2B + AA + Bismuth + PEPPS	91% (n=11)	16.91

PEPPS, potency-enhanced polyanionic phyto-saccharide; PPI, proton pump inhibitor (omeprazole); AA, antacids; H2B, histamine-2 blocker (ranitidine or cimetidine); bismuth, bismuth preparations.

Table 3. T-statistic value comparing PEPPS alone to PEPPS-enhanced treatments in horses.

Treatment with PEPPS alone compared to	t-Statistic	Degrees of freedom	2-tailed p value
PPI + PEPPS	0.289	87	0.773
PPI + AA + PEPPS	0.244	88	0.808
H2B + AA + PEPPS	1.177	106	0.242
H2B + AA + Bismuth + PEPPS	0.804	45	0.426

PEPPS, potency-enhanced polyanionic phyto-saccharide; PPI, proton pump inhibitor (omeprazole); AA, antacids; H2B, histamine-2 blocker (ranitidine or cimetidine); bismuth, bismuth preparations.

Table 4. Horses prior to treatment and day 14 to 20.

Treatment regimen	Number (%) with gastric ulcers	
	Prior to treatment	Day 14-20 on treatment
PEPPS alone treatment	8/8 (100)	0/8 (0)
PEPPS-augmented treatments	15/15 (100)	0/15 (0)

PEPPS, potency-enhanced polyanionic phyto-saccharide; Treatments augmented with PPI, proton pump inhibitor (omeprazole); AA, antacids; H2B, histamine-2 blocker (ranitidine or cimetidine); bismuth, bismuth preparations.

that a large majority of the horses were observed to improve better than 80% of the study's equine veterinarians would have thought possible, based on their collective past clinical experience. The historical experience of each veterinarian and their consensus of what constitute a significant deviation from that experience are subjective points. As a result, the data offers little predictive value and is mute as to efficacy. The study design provides an affirmative proof-of-principle supporting a plausible utility of PEPPS in the management of ulcers, diarrhea and colic associated with ulcers and diarrhea.

Another disadvantage of this study is that the manner of recruitment gives rise to bias. Practitioners were self-selected by virtue of responding to advertisements regarding a new gastrointestinal protectant which is resold at profit if the product is prescribed to a patient. Data obtained utilizing this method of recruitment is

vulnerable to a self-selection bias that is profit driven. In general, an appropriately randomized, placebo-controlled blinded investigation would best quantify the efficacy of PEPPS and thereby provide a better basis on which to predict the benefit of PEPPS in managing dysfunctional GI symptoms.

Strengths of this observational study

Despite limitations on study design, an observational study such as this can provide "real world" setting information useful in evaluating the comparative effectiveness of PEPPS (Dreyer et al., 2010). From this trial, there appear to be positive benefits associated with the use of PEPPS in managing ulcers, diarrhea and colic in horses due to ulcers and diarrhea.

Firstly, the geographic diversity of state-licensed veterinarian was a significant factor. Eighty percent of the equine regions in the US are represented in this study. The data reflected a national experience involving equine practitioners licensed in 20 of the top 25 states, with the highest horse population and ownership. The high percent response associated with PEPPS was uniform. This implies that positive association of PEPPS with symptom resolution was universal with no significant variability due to difference in region or physician practice.

Secondly, the association of a positive outcome with PEPPS demonstrated no deference to the age of the horse. All ages of horses responded similarly. The horse's age did not alter response to PEPPS. Clinical response was high across all age groups involved, with foals, yearlings and older horses responding equally well to PEPPS.

Thirdly, though horses could have improved in the same timeframe on their existing treatments, it was not likely. The continuation of pre-existing ulcer treatments, to which PEPPS was added, had no added advantage of either treatment over each other. Positive outcomes for ulcer colic in horses on PEPPS alone (81%, 29/36) was indistinguishable from the outcomes in horses treated with omeprazole, antacid and PEPPS. In this study, there did not appear to be any advantage over using PEPPS alone, as 30/37 (81%) horses had virtually the same outcome using PEPPS alone versus PEPPS with the other treatments. Prior to use of PEPPS, the other treatment regimens were failing. The time horses were on the failing treatments was not assessed, however given that the study was conducted within a practice setting, it is assumed initial treatments fail a reasonable test of time, prompting the veterinarians to prescribe PEPPS as an additional therapy.

There did not appear to be any combination of omeprazole, ranitidine or antacid that provided a discernable pre-existing advantage or disadvantage. PEPPS alone was associated with an 81% (29/36) positive outcome, not perceptibly different than omeprazole + antacids + PEPPS at 81% (30/37) or omeprazole + PEPPS at 83% (44/53). Though the positive outcome associated with ranitidine + antacid + PEPPS, 88% (63/72) was greater than that of using PEPPS alone, [81% (29/36)]; the author is unaware of any mechanism of action related to acid reduction by which ranitidine and antacids would be superior to omeprazole which was associated with lower positive outcome.

Using PEPPS alone at the onset of illness was associated with positive outcome equivalent to adding PEPPS to regimens deemed by the veterinarians to have failed. In the practice setting, failing treatments (that are not causing harm) are continued, as the practitioner does not know for sure the possible benefits that suboptimal treatments may be providing, but will prescribe an additional treatment (PEPPS) in hopes of expediting

clinical improvement. There was no statistically significant difference in using PEPPS alone versus using PEPPS with omeprazole, ranitidine, antacids or bismuth. This observation held true despite the diverse geographic regions of the country and the varying recreational uses of the horses. Additionally, the extended study period of 3.5 years averaged in the effects on positive outcomes, if any, attributable to seasonality.

Use of PEPPS associated with accelerated healing of ulcers without acid inhibition

Complete healing occurred within 14 to 20 days using either PEPPS alone (n = 8) or PEPPS in addition of clinically ineffective treatments (n = 15) in 23 horses with endoscopic evidence of gastric ulcers. This is 8 to 14 days sooner than the 28 days required for omeprazole to achieve statistically significant healing (Merial, 1999). Healing independent of acid treatment was reported in the Merial study (1999), wherein nearly 40% of horses on placebo either improved or were completely healed, giving support to the notion of an acid-independent ulcer-deterrent system within the mucosa. While 15 of 23 horses with ulcers were on acid reduction therapies with PEPPS, 8 of 23 were on PEPPS alone healed sooner that would otherwise be expected. Acid-mitigating therapies have no known direct interaction with mucosal mechanisms responsible for healing ulcers, but muco-adherent PEPPS does. Accelerated healing in 14 to 20 days was a positive outcome associated with the use of PEPPS alone in horses with ulcers.

Plausible mechanism of action for PEPPS

Slippery elm mucilage is a polyanionic phytosaccharide similar to sucralfate, in that it is a multi-saccharide chain that contains branched substituents with a high electro-negative density. In slippery elm, electro-negative galacturonic acid corresponds to the electro-negative sulfates of sucrose disaccharide of sucralfate. When the elm polysaccharide is placed in a solution of cations and multi-dentate chelators, it is assumed that, similar to other saccharides (and sucralfate), it polymerizes into a layered macromolecule with egg-box type configuration (DeKerchove and Elimelech, 2007; Grant et al., 1973). This layered configuration potentiates the saccharide's electronegative density, augments its muco-adherence (Dobrozsi et al., 1999; Jubeh et al., 2004) and increases its surface concentration (McCullough, 2010). It is assumed that as such a polymer, PEPPS exhibits cyto-protective characteristics reportedly existing with sucralfate. That is, similar to sucralfate, the muco-adherence of PEPPS is indifferent to gastric acidity (Steiner et al., 1982), and its strength of binding, unaffected by pH (Danesh et al., 1988).

Additionally, by virtue of its electro-density, PEPPS binds enteric growth factors as does sucralfate (Jones et al., 2004; Konturek et al., 1991, 1995) and thereby facilitates immediate mucosal regeneration (Tarnawski et al., 1986). Like sucralfate, it is supposed that the electronegative density of PEPPS enables it to modulate voltage-gated nociceptors of the enteric mucosa (Slomiany et al., 1992), thereby repolarizing neural fibers of the vagus nerve reversing colic and pain sensation from acid (Holtzer, 2001, 2004), nausea and colic (Beyak and Grundy, 2005). Just as high doses of sucralfate (25 to 100 mg/kg/dose) by some mechanism stop diarrhea in small animals in 2 to 4 doses (Steiner, 1990), so does PEPPS in the same manner relieve diarrhea in the horse. While the exact mechanism of action of PEPPS, and for that matter, of sucralfate, is not entirely clear, these comments serve as a start for discussion and future analysis. In all, the mechanism of action utilized by PEPPS allows the management of symptoms and signs via direct engagement of enteric elements responsible for mucosal integrity and normative function of the GI tract.

Conclusion

The majority of horses treated with PEPPS for colic due to ulcer and diarrhea were observed to have shortened clinical course to an extent not anticipated by experienced equine practitioners. This data was generated by veterinarians practicing in 20 out of 25 most populous equine owning states. While all equine patients eventually responded to PEPPS, a majority of horses responded within 4 days or 7 doses, returning to baseline feeding habits, demeanor and bowel habits. Additionally, whenever PEPPS was used, gastroscopy-positive ulcerations healed within 14 to 20 days. This was true in eight horses that healed using PEPPS alone without acid mitigating therapies. The data from this study supports the notion that PEPPS may be useful in the management of equine ulcer, colic and diarrhea. Yet blinded, randomized placebo-controlled trials are needed to assess the true efficacy of potency-enhanced polysaccharides.

ACKNOWLEDGEMENT

Fieldwork and data collection were funded as part of Mueller Medical International LLC research on polyanionic saccharides as *in vivo* surface active agents for epithelial mediated processes.

REFERENCES

American Horse Council (2005). www.horsecouncil.org/national-economic-impact-us-horse-industry.
Andrews F (2005). Ulcers in the Stomach and Colon; Diagnosis and

Treatment: A Pain in the Gut!
www.ivis.org/proceedings/aaepfocus/2005/Andrews.pdf.
Beyak MJ, Grundy D (2005). Vagal afferents innervating the gastrointestinal tract. In: Bradley JU, Daniel W (eds.), *Advances in Vagal Afferent Neurobiology*, CRC Press, Chap 12 pp. 315-350.
Danesh BJZ, Duncan A, Russell RI, Mitchell G (1988). Effect of intragastric pH on mucosal protective action of sucralfate. *Gut* 29(10):1379-1385.
DeKerchove AJ, Elimelech M (2007). Formation of polysaccharide gel layers in the presence of Ca²⁺ and K⁺ ions: Measurements and mechanisms. *Biomacromolecules* 8(1):113-121.
Dreyer NA, Tunis SR, Berger M, Ollendorf D, Mattox P, Gliklich R (2010). Why observational studies should be among the tools used in comparative effectiveness research. *Health Affairs* 29(10):1-8.
Dobrozsi DJ, Smith RL, Sakr AA (1999). Comparative mucoretenion of sucralfate suspensions in an everted rat esophagus model. *Int. J. Pharm.* 189(1):81-89.
Galvin N, Dillon H, McGovern F (2004). Right dorsal colitis in the horse: Mini review and reports on three cases in Ireland. *Irish Vet. J.* 57(8):467-473.
Grant GT, Morris ER, Rees DA, Smith RJC, Thom D (1973). Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Lett.* 32(1):195-198.
Hannan EL (2008). Randomized clinical trials and observational studies. State-of-the-art paper. *J. Am. Coll. Cardiol. Interv.* 1(3):211-217.
Hammond CJ, Mason DK, Watkins KL (1996). Gastric ulceration in mature thoroughbred horses. *Equine Vet. J.* 18:284-287.
Hillyer MH, Taylor FG, French NP (2001). A cross-sectional study of colic in horses on thorough bred training premises in the British Isles in 1997. *Equine Vet. J.* 33(4):380-385.
Holzer P, Michil T, Danzer M (2001). Surveillance of the gastrointestinal mucosa by sensory neurons. *J. Phys. Pharm.* 52:505-521.
Holzer P (2004). Gastrointestinal pain in functional bowel disorders: Sensory neurons as novel drug targets. *Expert Opin. Ther. Targets* 8:107-123.
Jones LS, Yazzie B, Middaugh CR (2004). Polyanions and the proteome. *Mol. Cellul. Proteomics* 3:746-776.
Jones MK, Tomikawa M, Mohajer B, Tarnawski AS (1999). Gastrointestinal mucosal regeneration: Role of growth factors. *Front. Biosci.* 15(4):303-306.
Jubeht TT, Barenholz Y, Rubinstein A (2004). Differential adhesion of normal and inflamed rat colonic mucosa by charged liposomes. *Pharmaceut. Res.* 21(3):447-453.
Kaneene JB, Ross WA, Miller R (1997). The Michigan equine monitoring system. II. Frequencies and impact of selected health problems. *Prev. Vet. Med.* 29(4):277-292.
Knudson A (2009). Stomach ulcers identified in 92% of racing horses. Nationwide events demonstrate all horses at risk for stomach ulcers. *Merial News Release*. (<http://www.equinechronicle.com/health/checking-for-equine-stomach-ulcers-what-horse-owners-need-to-know.html>)
Konturek JW, Brzozowski T, Konturek SJ (1991). Epidermal growth factor in protection, repair, and healing of gastroduodenal mucosa. *J. Clin. Gastroenterol.* 131:S88-S97.
Konturek SJ, Konturek JW, Brzozowski T, Slomiany BL, Slomiany A (1995). Effects of sucralfate on growth factor availability. In: Hollander D, Tygat GNJ (eds.), *Sucralfate: From basic science to the bedside*, Plenum Publishing Corporation, pp. 175-189.
Mairs TS, Divers TJ, Ducharme NG (2002). *Manual of Equine Gastroenterology*. Saunders WB, Harcourt Publishers Ltd. Elsevier Sci. Edinburgh. pp. 1-540
Martinson K, Hathaway M, Wilson JH, Gilkerson B, Peterson PR, Delvecchio R (2006). University of Minnesota horse owner survey. *Ext. J. Res.* Brief 44(6):1-12.
McCullough RW (1995). Gastrointestinal anti-irritant composition comprising sucralfate and methods of use. U.S. Patent 5,447,918.
McCullough RW (2010). Saccharide compositions and method of use. U.S. Patent 7, 795, 239.
McCullough RW (2012). Immuno-modulators for treating functional epithelial syndromes. Provisional Application 61/673,351.
Merial (1999). US FDA Freedom of Information Summary Gastrogard®

- (omeprazole) NADA Number 141-123.
- Murray MJ, Schusser GF, Pipers FS, Gross SJ (1996). Factors associated with gastric lesions in thorough bred racehorses. *Equine Vet. J.* 28:368-374.
- Pai R, Tarnawski A (1998). Signal transduction cascades triggered by EGF receptor activation: Relevance to gastric injury repair and ulcer healing. *Dig. Dis. Sci.* 439:145-225.
- Skov OP (1988). Role of epidermal growth factor in gastroduodenal mucosal protection. *J. Clin. Gastroenterol.* 101:S146-S151.
- Slomiany BL, Liu J, Slomiany A (1992). Modulation of gastric mucosal calcium channel activity by sucralfate. *Biochem. Int.* 28(6):1125-1134.
- Steiner K, Buhring KU, Faro HP, Garbe A, Nowak H (1982). Sucralfate: Pharmacokinetics, metabolism and selective binding to experimental gastric and duodenal ulcers in animals. *Arzneimittelforschung* 32(5):512-518.
- Steiner K (1990). Use of sucralfate for controlling emesis and/or diarrhea. US Patent 4,945,085.
- Szabo S (1991). The mode of action of sucralfate: the 1 x 1 x 1 mechanism of action. *Scand. J. Gastroenterol. Suppl.* 185:7-12.
- Szabo S, Folkman J, Vattay P (1994). Accelerated healing of duodenal ulcers by oral administration of a mutein of basic fibroblast growth factor in rats. *Gastroenterology* 106(4):1106-1111.
- Tams TR (2003). *Handbook of Small Animal Gastroenterology*. WB Saunders. Elsevier Science (USA) St Louis.
- Tarnawski A, Hollander D, Krause WJ, Zipser, RD, Stachura J, Gergely H (1986). Does sucralfate affect the normal gastric mucosa? Histologic, ultrastructural, and functional assessment in the rat. *Gastroenterology* 90(4):893-905.
- Tarnawski A, Hollander D, Stachura (1987). Effect of sucralfate on the normal human gastric mucosa. Endoscopic, histologic, and ultrastructural assessment. *Scand. J. Gastroenterol. Suppl.* 127(22):111-123.
- Tarnawski A, Jones K (1998). The role of EGF and its receptor in mucosal protection, adaptation to injury and ulcer healing. Involvement of EGF-R signal transduction pathways. *J. Clin. Gastroenterol.* 27:S12-20.
- Tinker MK, White NA, Lessard P (1997). Prospective study of equine colic incidence and mortality. *Equine Vet. J.* 29(6):448-453.
- Translational Medicine Research Center at Mueller Medical International (2002). Animal data from US veterinarian experience.
- Traub-Dargatz JL, Koprak CA, Seitzinger AH, Garber LP, Forde K, White NA (2001). Estimate of the national incidence of and operation-level risk factors for colic among horses in the United States, Spring 1998 to Spring 1999. *J. Am. Vet. Med. Assoc.* 219(1):67-71.
- Upton R, Axentiev P, Swisher D (2011). *American Herbal Pharmacopoeia and Therapeutic Compendium. Monograph on Slippery Elm Inner Bark – Ulmus Rubra Muhl.* pp. 1-40.
- USDA (2001a). Animal and Plant Health Inspection Service. Incidence of Colic in US Horses.
- USDA (2001b). Animal and Plant Health Inspection Service. National Economic Cost of Equine Lameness, Colic and Equine protozoal myeloencephalitis (EPM) in the United States.
- Vatistas NJ, Snyder JR, Carlson G, Johnson B, Arthur RM, Thurmond M, Lloyd KCK (1994). Epidemiological study of gastric ulceration in the Thoroughbred race horse: 202 horses 1992-1993. 40th AAEP Convention Proceedings, pp. 125-126.
- Veterinary Desk Reference (2011). Thorne Vet. <http://viim.org/veterinarians-desk-reference.php>.
- Ward M (2010). *Natural Ulcer Relief for Horses*. M Ward DVM copyright Austin TX. http://www.holistichorsekeeping.com/resources/articles/natural_ulcer.html.

Full Length Research Paper

Prevalence of bovine coccidia in Kombolcha district of South Wollo, Ethiopia

Alula Alemayehu^{1*}, Mohammed Nuru¹ and Timketa Belina²

¹School of Veterinary Medicine, Wollo University, Dessie, Ethiopia.

²College of Veterinary Medicine Harar, Haramaya University, Ethiopia.

Accepted 8 February, 2013

A cross sectional study was conducted from November 2011 up to April 2012 in Kombolcha town to determine the prevalence of coccidia infection in calves. Fecal samples were collected from a total of 288 calves with the age of 1 month to 1 year old which were included in the study purposively. After collection, the samples were transported to the laboratory and examined for the presence of *Eimeria* oocyst by flotation techniques. For positive sample, a solution of 2.5% potassium dichromate ($K_2Cr_2O_7$) was added to the feces containing the oocyst for sporulation and identification of the species. Out of 288 calves, 92 (31.9%) were found to be positive for *Eimeria* species. There was statistically significant difference ($P < 0.05$) in the prevalence of coccidia infection to different age of animals or fecal consistency. However, the difference was not statistically significant ($P > 0.05$) between coccidia infection and sex, breed, body condition, address or management system. Five species of *Eimeria* were identified in the study and the most prevalent species were *Eimeria bovis* (42.3%), *Eimeria zuernii* (28.3%) and *Eimeria auburnensis* (13.0%). The other species were *E. ellipsoidalis* (8.7%), *E. alabamensis* (4.3%) and unidentified oocyst (3.3%). In conclusion, the present finding has demonstrated that bovine coccidia are one of the important pathogens in calves in the study area. Further epidemiological investigations are required to determine the *Eimeria* species composition and different agro ecological risk factor on the occurrence of the disease.

Key words: Calves, coccidiosis, *Eimeria*, Kombolcha, prevalence, risk factors.

INTRODUCTION

Coccidiosis is a parasitic disease of the intestinal tract caused by microscopic organisms called coccidia and is one of the most common and important disease of cattle worldwide. Bovine coccidiosis has been observed in almost all areas where cattle are raised and is usually the most common and important in calves younger than 1 year. All calves managed under conventional systems are exposed to coccidia and become infected early in life. Many studies indicated that under natural conditions, mixed species infections are much more common than mono species infection (Ernst et al., 1987). Coccidiosis in

cattle is particularly a problem of confined animals kept under intensive husbandry practices. The disease is more common in housed animals than in those on pastures. In associations with other enteropathogens, coccidia has been indicated as an important cause of diarrhea in calves (Radostits et al., 1994). Coccidiosis spreads from one animal to another by contact with infected feces and is one of the most alarming problems for calf rearing industry. The most common clinical manifestations include inappetence, weakness, and loss of weight, diarrhea, depression and anemia (Soulsby, 1982). The development of clinical coccidiosis in cattle mainly depends on factors like species of *Eimeria*, age of infected animal, number of oocysts ingested, presence of concurrent infections and type of production system and management practices (Daugschies and Najdrowsk,

*Corresponding author. E-mail: alulaalemayehu@gmail.com.

Tel: +251912786586. Fax: +251331124352.

2005).

More than 13 species of *Eimeria* and one species of *Isospora* have been described to infect cattle and causes of coccidiosis. *Eimeria bovis* and *Eimeria zuernii* are considered the most pathogenic species as they are usually associated with clinical coccidiosis under field conditions. Thus, determination of prevalence, species composition, associated risk factors and animal management and husbandry practice is very useful in designing efficient control strategies (Ernst et al., 1984). *E. zuernii*, *E. bovis* and *Eimeria auburnensis* are the species most often associated with clinical disease in cattle, and other species have been shown to be mildly or moderately pathogenic. Coccidiosis is commonly a disease of young cattle (1 to 2 month to 1 year) and usually is sporadic during the wet seasons of the year (Fraser, 2006).

The prevalence, species composition, and importance of bovine coccidiosis have been documented in various countries of the world. Ernst et al. (1987) reported 82.28% infection rate in the coastal plain area of Georgia (USA); Rodriguez-Vivas et al. (1996) reported 87.8% infection rate in a sub humid tropical climate; Pandit (2009) reported 73.2% infection rate in Kashmir valley. In Ethiopia, Abebe et al. (2008) reported an overall prevalence of 68.1% in cattle in Addis Ababa and Debre Zeit area. However, there is lack of information on the occurrence and losses associated with bovine coccidiosis and very little attention has been given to the role of coccidiosis as the cause of disease and production losses in cattle in Ethiopia, especially in Kombolcha district of South Wollo. Therefore, taking into account the significance of the parasite as one of the most important causes of economic losses and the scarcity of information in the country, the present study was designed to determine the prevalence, species composition, and associated risk factors with *Eimeria* infections of calves in Kombolcha district.

MATERIALS AND METHODS

Study area

The study was conducted in Kombolcha town, which is found to the North East of Ethiopia in Amhara regional state from November, 2011 to April, 2012. The town is located in a range of altitudes between 1,500 and 1,840 m above sea level with average rainfall of 750 to 900 ml during the study period. Its annual temperature ranges from 25 to 30°C and the relative humidity of the region varies from 23.9 to 79% (NMSA, 2010).

Study animals

The study animals were calves within the age of 1 month to 1 year old. A total of 288 fecal samples were collected and examined for coccidia species from different dairy farms and small holders found in Kombolcha. Examined animals were categorized into two age groups as group I = 1 to 6 months age and group II = >6 to 12 months age which was determined by asking the owner of the

animal orally (Abebe et al., 2008). Examined animals were also categorized into three according to their body condition: good, medium and poor. This is based on different body visible bone structure and fat deposit (Nicolson and Butterworth, 1986).

Sampling techniques and sample size determination

Purposive sampling technique was used to select the study animals from the target population. The sample size required for the study was determined according to Thrusfield (2005) as follows. By taking a 95% confidence interval, 20% expected prevalence whenever there is no information on the prevalence of the disease in the area and 5% desired absolute precision, the sample size was calculated as follows:

$$n = 1.96^2 \times P_{\text{exp}}(1 - P_{\text{exp}}) / d^2$$

where n = required sample size; P_{exp} = expected prevalence, d = desired absolute precision.

Therefore, 245 calves within the age of 1 month to 1 year old were required from target population in the study area. But, the sample size was maximized to 288 calves.

Experimental

Fecal sample collection

About 30 g fresh fecal sample was collected from rectum from each calf using sterile disposable plastic gloves. The samples were placed in a labeled clean plastic container (universal bottle) and were transported to the parasitological laboratory of Kombolcha Animal Health and Diagnostic Center on the same day of collection and were preserved at refrigerator until processing within 48 h of arrival. At the time of sampling, the name of the farm (owner), date of sampling, consistency of the feces (soft, watery or normal) and the age, sex, breed, address and management system were recorded for each calf on a data recording format.

Parasitological investigation

A 5 g portion of each of the 288 fecal samples collected from the total of 30 g was weighed out using a balance and put in a 50 ml beaker. 42 ml of water was added, mixed thoroughly and poured into a 100-ml glass beaker through a strainer. The 50-ml glass beaker was rinsed with 8 ml of water and the total fluid was poured into four 15-ml conical tip centrifuge tubes. After centrifugation at 1,500 rpm for 5 min, the supernatant was decanted and a sugar solution (specific gravity 1.25) was added to the sediment, until the tube was about half full. The content of each test tube was thoroughly mixed with a wooden applicator stick. With the aid of a conical flux, more sugar solution was added until a convex meniscus was formed on top of the tube. A glass cover slip was placed on top of each tube and was left for 30 min. Then, each glass cover slip was briskly lifted up and placed on a clean glass slide, not allowing formation of air bubbles. The entire area under each cover slip was examined under a binocular microscope at 400x magnification (Hendrix, 1998).

Sporulation of *Eimeria* oocysts

A solution of 2.5% potassium dichromate was added to each fecal sample, which contained most of the *Eimeria* oocyst in a beaker, mixed thoroughly with a wooden applicator and poured into a Petri dish. Each Petri dish was left on the bench in the laboratory to allow

sporulation. Thereafter, every 24 h, the culture of oocysts was mixed thoroughly and with the aid of medicine dropper, a drop of the culture was placed on a glass slide, covered with a glass cover slip and examined under the microscope to determine when sporulation occurred. When sporulation of oocysts was completed after 14 days, the Petri dish containing oocysts was covered up and stored in a refrigerator at 5°C until needed. Identification of coccidian species will be based on the morphological features of the sporulated oocysts (size, shape, color, and texture of oocyst wall, presence or absence of micropyle, polar cap) and time of sporulation with the aid of taxonomic keys (Soulsby, 1982; Kennedy and Kralkara, 1987; Sommer, 1998).

Data management and analysis

Data collected from study sites were entered and stored in a Microsoft excel spread sheet program and coded for analysis. Statistical analysis was done on Statistical Package for Social sciences (SPSS) 17.0 statistical software. The prevalence was calculated for all data as the number of infected individuals divided by the number of sampled individual and multiplied by 100. Categorical data were analyzed first with the chi square (χ^2) test for independence as a screening process. A P-value < 0.05 was considered as statistically significant.

RESULTS

Prevalence and analysis of potential risk factors for the occurrence of bovine coccidia

Out of all 288 samples, a total of 92 samples (31.9%) tested positive for *Eimeria* species oocysts as shown in Table 1.

Analysis of the potential risk factor for the occurrence of coccidia has revealed that there were a significant association ($P < 0.05$) with age and fecal consistency of the calves. However, there is no significant association ($P > 0.05$) with breed, sex, body condition, address management system of the calves and coccidia (Table 1).

Percentage and analysis of *Eimeria* spp. with the suspected risk factors

Out of 288 calves whose fecal samples were examined, 92 (31.9%) were found to be positive for 5 species of *Eimeria*. The most prevalent species were *E. bovis*, which occurred in 39 (42.3%), *E. zuernii* in 26 (28.3%), *E. auburnensis* in 12 (13.0%), *Eimeria ellipsoidalis* in 8 (8.7%), *Eimeria alabamensis* in 4 (4.3%) and unidentified oocyst were observed in 3 (3.3%) (Table 2).

Analysis of the potential risk factors and species of *Eimeria* has revealed that there is a significant association ($P < 0.05$) between the occurrence of *E. bovis* with body condition and fecal consistency. In addition, fecal consistency has a significant association ($P < 0.05$) for the occurrence of all *Eimeria* spp. However, there is no significant difference ($P > 0.05$) between sex, breed, age, and address and management system for the

occurrence of *Eimeria* spp.

DISCUSSION

The present study has revealed that the presence of bovine coccidia species parasitizing the gastro intestinal tract of calves under the age of one years in Kombolcha district of South Wollo. The overall prevalence of *Eimeria* spp. is 31.9%, which is lower than previous findings reported in Addis Ababa and Debre Zeit by Abebe et al. (2008) (68.1%), in the coastal plain area of Georgia (USA) by Ernst et al. (1987) (82.28%) and in sub-humid tropical climate by Rodriguez-Vivas et al. (1996) (87.8%). This variation is most likely attributed to the differences in agro-ecology, and husbandry practices of the study animals in different countries (Radostits et al., 2006).

Analysis of risk factor in the association of disease occurrence has revealed that there was no statistically significant association ($P > 0.05$) between breed and coccidia infection. These indicate that body condition does not have influence on the occurrence of coccidia infection. This is due to either equal chance of accessing the oocysts or no difference on protective immunity for the disease. This finding agrees with the report of Abebe et al. (2008). There was no statistically significant association ($P > 0.05$) between sex and coccidia infection. The prevalence in female calves was similar to that of males in this study. This finding agrees with the report of Abebe et al. (2008). There was no statistical significant association ($P > 0.05$) between the address of the animals and coccidian infection. However, the present study disagrees with previous studies indicating that there was a statistical significant association between geographic zone and the occurrence of coccidian infection (Abebe et al., 2008).

There was a strongly significant association ($P < 0.05$) between the age of the calves with the risk of infection in which the prevalence of coccidia appeared to follow an age pattern. Higher infection rate was observed in calves >6 to 12 months of age than calves of 1 to 6 months of age due to the fact that there was good nursing of the colostrum feeding for younger calves. During investigation, almost all the calves older than 6 months were housed in overcrowded condition, less care were given and have easy contact with adult animals. This has given more chance for the animals to lick each other and ingest large number of oocysts, which is in agreement with previous reports (Kennedy, 2001; Abebe et al., 2008; Rodriguez-Vivas et al., 1996; Radostits et al., 2006). Coccidiosis occurs most commonly in young animals with a seasonal incidence when young calves are brought together for weaning or moved into feedlots or fed in small areas for the winter months. The prevalence of infection and the incidence of clinical disease are also age related (Radostits et al., 2006).

There was no a statistical significant association between body condition of the animals and coccidian

Table 1. Prevalence and analysis of potential risk factors for the occurrence of bovine coccidian.

Risk factor	No. of calf examined	No. of positive	Prevalence (%)	χ^2	P- value
Breed					
Local	162	53	32.7	0.105	0.750
Cross	126	39	31.0		
Sex					
Male	103	31	30.1	0.252	0.616
Female	185	61	33.0		
Address					
Urban	137	44	32.1	0.004	0.952
Rural	151	48	31.8		
Age					
1-6 month	112	27	24	5.178	0.023
>6-12month	176	65	36.5		
Body condition					
Good	114	34	29.8	0.698	0.705
Medium	106	37	34.9		
Poor	68	21	30.9		
Faecal consistency					
Normal	176	18	10.2	102.278	0.00
Soft	62	36	58.8		
Diarrheic	50	38	76.0		
Management					
Intensive	109	38	34.9	0.687	0.407
Extensive	179	54	30.2		
Total	288	92	31.9%		

Table 2. Percentage of *Eimeria* spp. distribution in Kombolcha district.

Species of <i>Eimeria</i>	Number of positive sample	Prevalence (%)
<i>E. bovis</i>	39	42.4
<i>E. zuernii</i>	26	28.3
<i>E. auburnensis</i>	12	13.0
<i>E. ellipsoidalis</i>	8	8.7
<i>E. alabamensis</i>	4	4.3
Unidentified oocyst	3	3.3
Total	92	100

infection ($P>0.05$). These indicate that body condition does not have influence on the occurrence of coccidia infection. This is due to either the level of infection, sampled size or most of the affected animals harbor the disease without showing clinical signs (Fraser, 2006). There was statistically significant ($P<0.005$) difference in

prevalence rate between fecal consistency with coccidia infection which agrees with the finding of Pandit (2009). However, this finding disagrees with the report of Abebe et al. (2008). In the present study, 76.0% (38/50) of diarrheic calves (blood stained, watery and fetid diarrhea) were found to be positive for *Eimeria*. However, there

were no apparent clinical signs in most of the animals sampled for the study. The influence of management system on prevalence of coccidia has revealed that there was no statistically significant association between them ($P>0.05$). This finding disagrees with the previous reports by Abisola (2004) and Kennedy and Kralka (1987). This might be attributed to the fact that hygienic system of the barn, nutritional status, contamination of the feed or overcrowding of the animal was similar in both management systems.

The overall prevalence of *Eimeria* spp. in this study was 31.9% (Table 2) and the most prevalent among the 5 species encountered were *E. bovis* (42.4%), *E. zuernii* (28.3%) and *E. auburnensis* (13.0%). These species are the most frequently reported *Eimeria* spp. in outbreaks of coccidiosis throughout the world (Andrews et al., 2004; Abebe et al., 2008; Ernst et al., 1987; Kasim and Al-Shawa, 1985; Oda and Nishida, 1990). This high prevalence of pathogenic species (*E. bovis* and *E. zuernii*) in infected calves and the greater proportions of subclinical infections could negatively influence animals' productivity and cause economic losses from poor feed efficiency, slow weight gain, weight loss, failure to grow to their full potential, and increased susceptibility to other diseases (Fraser, 2006). Moreover, continuous oocysts shed from subclinical infected calves contaminate the environment or the hair coats and cause severe coccidiosis in highly susceptible new calves that are kept in these areas (Abebe et al., 2008; Radostits et al., 2006).

Conclusion

This study has revealed that the prevalence of calves *Eimeria* infection in Kombolcha district was 31.9% and five *Eimeria* spp. namely *E. bovis*, *E. zuernii*, *E. auburnensis*, *E. ellipsoidalis* and *E. alabamensis* were identified in all *Eimeria* positive fecal samples. The high prevalence of *Eimeria* spp. was considered as one of the important infection in cattle farms in the study area. The prevalence of coccidia has no significant association with address, sex, breed, body condition, management system of animals examined during the study period. However, the disease has a significant association ($P<0.05$) with age and faecal consistency of the calf. Results from this study indicate the *Eimeria* infection has a great significance for the livestock producer and need a serious control and preventive issue. Therefore, further epidemiological investigation on coccidia species should be needed in the study area.

ACKNOWLEDGEMENT

We would like to forward our appreciation for staff members of Kombolcha Regional Veterinary Laboratory for their cooperation to accomplish this work.

REFERENCES

- Abebe R, Kumesa B, Wessene A (2008). Epidemiology of *Eimeria* infections in calves in Addis Ababa and Debre Zeit Dairy Farms, Ethiopia. Intern. J. Appl. Res. Vet. Med. 6:24-30.
- Abisol TO (2004). Studies on Bovine Coccidia in Parts of Plateau State of Nigeria. MSc. Thesis, Nigeria.
- Andrews AH, Blowey RW, Boyd H, Eddy RG (2004). Bovine Medicine. 2nd Ed. Blackwell Science Ltd., Oxford, UK, pp. 282-283.
- Dauguschies A, Najdrowsk M (2005). Eimeriosis in cattle: Current understanding. J. Vet. Med. 52:417-427.
- Ernst JV, Ciordia H, Stuedeemann JA (1984). Coccidia in cows and calves on pasture in South Georgia (USA). Vet. Parasitol. 15: 213-221.
- Ernst JV, Stewart TB, Witlock DR (1987). Quantitative determination of coccidian oocysts in beef calves from the coastal plain area of Georgia (USA). Vet. Parasitol. 23: 1-10.
- Fraser CM (2006). The Merck Veterinary Manual, A Hand Book of Diagnosis Therapy and Disease Prevention and Control for Veterinarians. 7th Ed., Merck and Co. Inc, Rahway, NIT, USA, pp. 714-717.
- Hendrix CM (1998). Diagnostic Veterinary Parasitology. 2nd Ed., St. Louis. Mosby Inc., pp. 239-264.
- Kasim AA, Al-Shawa YR (1985). Prevalence of *Eimeria* in feces of cattle in Saudi Arabia. Vet. Parasitol. 17:95-99.
- Kennedy MJ (2001). Coccidiosis in cattle. In: *AGRI FACTS*. monton, Alberta, Canada: Alberta Agriculture, Food and Rural Development, Government of Alberta.
- Kennedy MJ, Kalka RA (1987). A survey of *Eimeria* species in cattle in central Alberta. Can. Vet. J. 28:124-125.
- Nicolson MJ, Butterworth MH (1986). A guide to condition scoring of zebu cattle. International livestock center for Africa, Addis Ababa, Ethiopia.
- NMSA (2010). National Meteorology Service Agency. Kombolcha Branch, Kombolcha, Ethiopia.
- Oda K, Nashida Y (1990). Prevalence and distribution of bovine coccidia in Japan. J. Vet. Sci. 52: 71-77.
- Pandit BA (2009). Prevalence of Coccidiosis in Cattle in Kashmir valley. Vet. Scan. 4:16-20.
- Radostits OM, Blood DC, Gay CC (1994). Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses. 8th Ed., Bailliere Tindall, Philadelphia, pp.1181-1199.
- Radostits OM, Gay CC, Constable PD (2006). Veterinary Medicine. A Text Book of the Disease of Cattle, Horse, Sheep Pigs and Goats. 10th Ed., Sanders, Edinburgh, pp. 969-984.
- Rodriguez-Vivas RI, Dominguez-Alpizar JL, Torres-Acosta JF (1996). Epidemiological factors associated to bovine coccidiosis in calves (*Bos indicus*) in a sub humid tropical climate. Rev. Biomed. 7:211-218.
- Sommer C (1998). Quantitative characterization, classification and reconstruction of oocyst shapes of *Eimeria* species from Cattle. Parasitology 116:21-28.
- Soulsby EJM (1982). Helminths, Arthropods, and Protozoas of Domestic Animals. 7th Ed., Bailliere, Tindall and Cassell, London, pp. 594-664.
- Thrusfield M (2005). Veterinary Epidemiology. 3rd Ed., Blackwell Science Ltd., Oxford, UK, pp. 233-261.

Full Length Research Paper

Gross pathological changes in the reproductive tracts of cows slaughtered at two abattoirs in Southern Ethiopia

Berhanu Mekibib¹, Techan Desta² and Dawit Tesfaye^{1*}

¹School of Veterinary Medicine, Hawassa University, P. O. Box 05, Hawassa, Ethiopia.

²Mizan Agriculture and Rural Development Bureau, Mizan, Ethiopia.

Accepted 18 February, 2013

Abattoir based study was conducted to assess the type and prevalence of reproductive abnormalities and pregnancy status of cows slaughtered at Hawassa municipality abattoir and Tula slaughter house. Out of the 345 genital tracts examined, one or two gross abnormalities with different degrees of severity were observed in 124 (35.9%) of genital tract. The most common abnormalities encountered were ovariobursal adhesions (6.38%, n=22), endometritis (4.93%, n=17), and follicular cysts (4.35%, n=15). On anatomical basis, ovarian abnormalities (14.78%, n=51) were more frequent followed by uterine (10.43%, n=36), cervico-vaginal (7.25%, n=25) and oviductal (4.93%, n=17) abnormalities. Both breed and study area showed no statistically significant effect in the prevalence of reproductive abnormalities. Pregnancy was recorded in 26.67% (n=92) of the slaughtered cows, of which 45.65% (n=42) were in the first trimester, while 30.43% (n=28) and 23.91% (n=22) in the second and third trimesters, respectively. The current study revealed that reproductive tract abnormalities are important diseases in the study areas with considerable impact on the reproductive performance of cows. Moreover, the large number of cyclical (36.52%) and pregnant cows (26.67%) slaughtered without any gross abnormalities indicates the absence of proper gynecological examination prior to slaughtering.

Key words: Reproductive abnormalities, genital tract, slaughter, cows, Hawassa, Tula.

INTRODUCTION

The traditional milk production system dominated by indigenous breeds of low genetic potential for milk production accounts for 97 to 98% of the total annual milk production in Ethiopia (Felleke, 2003). This system is constantly challenged by shortage of feed, lack of capital with dairy producers, unimproved animal husbandry system; inefficient and inadequate milk processing skill and prevailing diseases including uterine diseases and anestrus.

Diseases and abnormalities of the female genital tract are believed to be the major reason for economic loss associated with infertility, culling and slaughtering of cows (Singleton and Dobson, 1995; Ashenafi, 2004; Abalti et al.,

2006). Since most reproductive tract problems lack additional outward manifestation, hence, examination of gross and microscopic lesions of genital tract play a central role in the identification of these problems. Most of these abnormalities can only be diagnosed when the animal is subjected to postmortem examination (Buregelt, 1997). Though, in different regions of Ethiopia, studies have been conducted on reproductive abnormalities of cows based on abattoir material; in Addis Ababa (Gebrekidan et al., 2009), in Sululta (Simenew et al., 2011), in Tigray (Zerihun, 2001), in Jimma (Amare, 2002), in Nekemte (Samuel, 2002) in Asela (Endalew, 2001), in Bahir Dar (Abalti et al., 2006) and in Awassa (Ashenafi, 2004). However, more systematic work has to be done to assess the problem in depth. Hence, the present study aimed to identify the possible causes of

*Corresponding author. E-mail: dawittesfa@gmail.com.

slaughtering of female cattle with due emphasis to reproductive organs and to establish the reproductive status (ovarian cyclicity) of slaughtered mature female cattle.

MATERIALS AND METHODS

Study area

The study was conducted at Hawassa Municipal abattoir and Tula slaughter house. Hawassa is the capital city of Southern Nations, Nationalities and Peoples Regional State (SNNPRS). The town is located at 275 km South of Addis Ababa with area coverage of 162,804 hectares. The total human population of the town is about 180,500 (CSA, 2004). The abattoir and slaughter house are located in Tula sub city at 5 and 18 km away from the town, respectively. Active and routine meat inspection is carried out in the abattoir to provide a wholesome meat to the residents of the city. Animals for slaughter at the abattoir are brought mainly from Hawassa, Shashamane, Arsi-Negelle, Dilla and Wolyta Soddo towns. However, cattle slaughtered at Tula slaughter house are not thoroughly inspected and destined for local consumption.

Study animals and design

The study animals included 345 cows (252 local, 58 Holstein Friesian and 35 cross) purchased from the aforementioned markets and brought to the abattoir for slaughtering purpose. All cows slaughtered at the municipality abattoir (n=95) and Tula slaughter house (n=250) during the six month study period were included in the study.

Experimental

Any relevant data about the productivity history like parity, reason for culling, breed, origin, and age of the slaughtered cows were collected from the owner to correlate with pathological finding of the reproductive organs. Age of the slaughtered cows was estimated according to the description given by De-Lahunta and Habel (1986). The genital tracts of all slaughtered cows were collected from both sources, placed in separate plastic bags and transported to Veterinary Parasitology and Pathology Laboratory, School of Veterinary Medicine, Hawassa University, for routine post-mortem examinations. The entire reproductive tract were visually examined and thoroughly palpated. Each reproductive tract was opened along the longitudinal axis starting from the vagina down to the horns and was observed for any abnormalities in color, odor, consistency and morphology (Feyissa and Bekana, 2000). Similarly, both ovaries were incised and inspected for any gross lesions. Moreover, the number of corpora albicantia and status of the corpus luteum (CL) were recorded. In pregnant tracts, the stage of pregnancy was determined based on crown rump length according to Evans and Sack (1973) and Elsayed et al. (1978) and was classified as early (<3 months), mid (3 to 6 months) and late (>6 months) using the formula for estimation of developmental stages (days) ($X = 2.5(y+1)$): where y is the crown-rump length in cm and X is developmental stages in days). Methylene blue dye was infused in to the oviduct using disposable syringe through infundibulum to check for non-visible in patent state of oviduct as described by Herenda (1987) and Assey et al. (1998).

Data management and analysis

The results obtained from gross examination of the reproductive

tracts of slaughtered cows were recorded on spreadsheet of Microsoft Excel and analyzed using STATA 9 statistical software. Simple descriptive statistics was used for the analysis of the data obtained. Categorical data were analyzed with the Chi-square (χ^2) test for independence. In all the analysis P value < 0.05 was considered for significance.

RESULTS

Abnormalities of the reproductive tracts

The overall abattoir prevalence of one or more reproductive abnormalities in cows of the two study sites was 35.9% (124/345). Out of these, eight cows were having two problems at a time. The prevalence of the problem by study sites and breed is summarized in Table 1. During the study period, the most frequently observed abnormalities or disease of the reproductive tract were ovariohysterectomy with an occurrence rate of 6.38% (n=22) followed by an ovarian cyst of 5.22% (n=18), endometritis of 4.93% (n=17), mucometra of 3.77% (n=13), vaginitis of 3.19% (n=11) and other less frequently observed abnormalities. Moreover, ovariohysterectomy, endometritis and follicular cyst were relatively more common in improved dairy cows than the local zebu (Table 2). Based on anatomical classification, abnormalities of the ovary (n=51, 14.78%) were found with highest occurrence rate followed by uterine (n=36, 10.43%), cervico-vaginal (n=25, 7.25%) and oviduct abnormalities 4.93% (n=345). Moreover, one case of macerated fetus was also observed (Table 3).

Pregnancy statuses

The total number of pregnant cows slaughtered during the study period in both study sites was 92. Of the total pregnancies, 45.65% (n=42) were found with early, 30.43% (n=28) were mid and 23.91% (n=22) were late stages. It was also found that the right ovaries had the highest physiological activities than the left ovaries and their percentage were 65.45 and 34.55% on the right and left, respectively.

Ovarian cyclicity

A total of 126 cows slaughtered during the study period were found cyclic. Based on the location, cyclicity was found to be 49.21, 45.24 and 5.56% on the right, left and both ovaries, respectively (Table 4).

DISCUSSION

The prevalence of reproductive tract abnormalities of cows recorded in the present study (35.94%) was in line with previous reports of Teklu (1999) (37.0%, Addis Ababa),

Table 1. Abattoir prevalence of reproductive tract abnormalities in cows (n=345).

Risk factor	Observation	Abnormality number	Prevalence (%)
Study area			
Hawassa	95	30	31.58
Tula	250	94	37.6
Breed			
Local	252	94	37.30
Holstein	58	19	32.76
Cross	35	11	31.45

Table 2. Prevalence of different reproductive abnormalities in different breeds.

Abnormality	Average age	Overall prevalence	Breed		
			Local	Holstein	Cross
Ovariobursal adhesion	7.2	6.38	5.56	8.62	5.71
Endometritis	6.7	4.93	4.36	5.17	8.57
Follicular cyst	6.8	4.35	3.97	5.17	5.71
Mucometra	7.8	3.77	4.76	1.72	-
Vaginitis	6.8	3.19	2.38	3.45	2.86
Hydrosalpinx	8.1	2.32	2.78	-	-
Cervicitis	7.2	2.32	1.19	5.17	-
Occluded oviduct	4.8	1.74	1.98	1.72	-
Parovarian cyst	7.1	1.74	1.98	1.72	-
Hydrometra	8.2	1.45	1.59	-	2.86
Ovarian hypoplasia/atrophy	6.5	1.16	1.59	-	-
Luteal cyst	6.3	0.87	0.79	-	2.86
Pyosalpinx	8.3	0.87	1.19	-	-
Tortuous cervical canal	9.5	0.58	0.79	-	-
Cervical ring hypoplasia	5	0.58	0.4	-	2.86
Vaginitis and cervicitis	5	0.58	0.79	-	-
Hypoplasia/atrophy of uterus	3	0.29	0.4	-	-
Macerated fetus	8	0.29	0.4	-	-

Ashenafi (2004) (33.3%, Hawassa), Abalti et al. (2006) (36.8%, Bahirdar) and Gebrekidan et al. (2009) (31.4%, Addis Ababa). However, it was higher than the reports of Abdissa (2000) (Bahirdar, 23.4%), Endalew (2001) (Assella, 26.3%), Zerihun (2001) (Raya valley, 22%), Samuel (2002) (Nekemte, 27.2%), Amare (2002) (Jimma, 29.2%), Assey et al. (1998) (Tanzania, 9%) and Chaudhari and Paul-Bokko (2000) (Nigeria, 12.5%). This variation could be due to the difference in geographical variation, sample size, breed, feed and health management of animals included in the study.

Ovariobursal adhesion (6.38%, n=22) was the most common reproductive abnormality encountered during the study followed by ovarian cyst (5.22%, n=18), endometritis (4.93%, n=17), mucometra (3.77%, n=13), vaginitis (3.19%, n=11). In line with this finding, Abalti et

al. (2006) encountered ovariobursal adhesion, endometritis and cystic ovaries as the most common abnormalities with respective prevalence of 5.5, 3.9 and 3.5%. Although, the exact mechanism by which adhesions develop is unclear (Roberts, 1986a, b), extreme adhesions have probably resulted from pregnancy complications that include retained fetal membranes and endometritis (Lewis, 1997). Mild adhesions could result from non-infectious conditions such as physical trauma as a result of rough manipulation of the genital tract during pregnancy diagnosis (Bondurant, 1999). Uterine infections were common findings in cows that had given birth with possible complications especially dystocia and retained fetal membrane.

Examination of ovarian cyclicity revealed 36.52% cyclic ovaries, which was lower than the report made by

Table 3. Prevalence of anatomical reproductive tract abnormalities of cows (n=345).

Abnormality	Occurrence rate (%)	Proportional frequency (%)
Ovarian abnormalities	14.78 (n=51)	
Ovariobursal adhesion	6.38	
Ovarian cyst	5.22	38.66
Parovarian cyst	1.74	
Ovarian hypoplasia/atrophy	11.45	
Uterine abnormalities	10.43 (n=36)	
Endometritis	4.93	
Mucometra	3.77	27.27
Hydrometra	1.45	
Hypoplasia/atrophy of uterus	0.29	
Cervico- vaginal abnormalities	7.25 (n=25)	
Vaginitis	3.19	
Cervicitis	2.32	
Cervicitis and vaginitis	0.58	18.94
Cervical ring hypoplasia	0.58	
Tortuous cervical canal	0.58	
Oviduct abnormalities	4.93 (n=17)	
Hydrosalpinx	2.32	
Occluded oviduct	1.74	12.88
Pyosalpinx	0.87	
Miscellaneous	0.87 (n=3)	
Ovariobursal adhesion and hydrosalpinx	0.29	
Ovariobursal adhesion and ovarian hypoplasia	0.29	2.27
Macerated fetus	0.29	

Table 4. Status of ovarian activities and pregnancy.

Status	Right (%)	Left (%)	Bilateral (%)
Cyclic (n=126)	49.21 (n=62)	45.24 (n=57)	5.5 (n=7)
Pregnancy (n=92)	65.45	34.55	-

Chaudhari and Paul-Bokko (2000) in Nigeria (55.49%), Samuel (2002) in Nekemte (65.55%), Amare (2002) in Jimma (62.2%) and Ashenafi (2004) in Hawassa (66.7%). This variation could be attributed to difference in breed, sample size and management. The presence of active corpus luteum in the right (49.21%), left (45.24%) and both ovaries (5.56%) was in agreement with the occurrence rate reported by Teklu (1999), who reported 55, 43, and 2% for the right, left and both ovaries, respectively. The result indicates that the right ovary is more physiologically active than the left ovary.

Pregnancy wastage (26.67%) noted in the present study was comparable with previous reports made by

Amare (2002) in Jimma (24.5%), Samuel (2002) in Nekemte (31.4%) and Ashenafi (2004) in Hawassa (29.91%). However, higher value was reported in East Africa Zebu by Assey et al. (1998) in Tanzania (54%), Abdissa (2000) in Bahirdar (37.8%) and Zerihun (2001) in Raya Valley (36.84%). Such high pregnancy wastage in slaughtered cows could be attributed to inadequate antemortem inspection of animals particularly on pregnancy diagnosis. Moreover, cattle owners in the area usually insist on sale of their pregnant cows, because of anticipated pregnancy failure or because of a comparably improved body condition during pregnancy. Though, it is difficult to estimate the total impact of slaughter of pregnant cows;

Chaudhari and Paul-Bokko (2000) concluded that pregnancy wastage accounts for 20 to 25% fall in livestock production particularly in the developing countries, especially sub-Saharan Africa.

Conclusions

Based on the findings of this study and personal communication with cattle owner, low milk yield, infertility and economic conditions were the major factors for slaughter of cows. More than 60% of cows were slaughtered while they were potentially fertile. Slaughtering of potentially fertile cows, that is, pregnant and cyclic cows, indicates the absence of proper antemortem pregnancy diagnosis and lack of record keeping about the different events of reproductive cycle. Coupled with the prevailing reproductive abnormalities, slaughtering of fertile cows cause huge fetal wastage, reduced replacement stock, pose selection pressure and other detrimental effects on the dairy industry. To combat this problem, complete antemortem examination, awareness creation to the owners about the causes and effects of the problem, enforcing laws guiding slaughter of cows are safely recommended.

REFERENCES

- Abalti A, Bekana M, Woldemeskel M, Lobago F (2006). Female genital tract abnormalities of Zebu cattle slaughtered at Bahir-Dar Town, North-Western Ethiopia. *Trop. Anim. Health Prod.* 38:505-510.
- Abdissa A (2000). A study on abnormalities of reproductive tract of the Zebu cows slaughtered in Bahradar, North West Ethiopia. Faculty of Veterinary Medicine AAU, Debre Zeit, Ethiopia, DVM thesis.
- Amare A (2002). A gross morphological study of genital organs form female Zebu cattle in and around Jimma town. Faculty of Veterinary Medicine AAU, Debre Zeit, Ethiopia, DVM thesis.
- Ashenafi D (2004). Study on gross genital abnormalities, pregnancy status and ovarian activity in Zebu cattle breeding slaughtered at Hawassa and Tula slaughter houses. Faculty of Veterinary Medicine AAU, Debre Zeit, Ethiopia, DVM thesis.
- Assey RJ, Kessy BM, Matovelo JA, Minga V (1998). Incidences of gross reproductive abnormalities in small East African Zebu Cattle. *Trop. Anim. Health Prod.* 30:361-368.
- Bondurant RH (1999). Inflammation in the bovine female reproductive tract. *J. Anim. Sci.* 77:101-110.
- Buregelt CD (1997). Color atlas of reproductive pathology of domestic animals. Mosby publisher, Philadelphia.
- CSA (2004). Central Statistics Authority, Livestock population of Ethiopia, central statistics authority, Addis Ababa, Ethiopia.
- Chaudhari SUR, Pau-Bakko B (2000). Reproductive status, pregnancy wastage and incidence of gross genital abnormalities in cows slaughtered at Maidughuri abattoir, Niger. *Pak. Vet. J.* 20:203-205.
- De-Lahunta A, Habel RE (1986). Teeth, Applied veterinary anatomy. W.B. Saunders Company. pp. 4-16.
- Elsayed MAI, Kessy BM, Maurya SN (1978). Developmental changes in the gravid uterus of the Tanzanian short horn Zebu cattle. *Alex. J. Vet. Sci.* 3:255-268
- Endalew G (2001). A study on gross abnormalities of genital organs of female cattle slaughtered in Asella town. Faculty of Veterinary Medicine AAU, Debre Zeit, Ethiopia, DVM thesis.
- Evans HE, Sack WO (1973). Prenatal development of domestic and laboratory mammal, growth curves, external features and selected references. *Anat. Histol. Embryol.* 2:11-45.
- Felleke G (2003). Milk and dairy products, post-harvest losses and food safety in Sub-Saharan Africa and the Near East. A review of the small scale dairy sector – Ethiopia. FAO Prevention of Food Losses Programme. FAO, Rome, Italy.
- Feyissa T, Bekana M (2000). A gross morphological abattoir study of genital organs from female crosses breed and Zebu cattle. Abstract of the 14th International Congress on animal reproduction, 2 - 6 July, Stockholm, Sweden. *Isr. J. Vet. Res.* 55:83-87.
- Gebrekidan B, Yilma T, Solmon F (2009). Major causes of slaughter of female cattle in Addis Ababa Abattoir enterprise, Ethiopia. *Indian J. Anim. Res.* 43:271-274.
- Herenda D (1987). An abattoir survey of reproductive organ abnormalities in beef heifers. *Can. Vet. J.* 28:33-36.
- Lewis GS (1997). Health problems of the postpartum cow, uterine health and disorders. *J. Dairy Sci.* 80: 984-994.
- Roberts SJ (1986a) Veterinary obstetrics and genital diseases. 3rd Ed., S.J.Roberts-Woodstock. pp 381-359.
- Roberts SJ (1986b). Infertility in the cow. In: Veterinary obstetrics and genital Disease, 2nd Ed., CBS publishers and Distributors, India. pp. 376-511.
- Samuel T (2002). Study on gross genital tract abnormalities of female Zebu cattle slaughtered in and around Nekemte town, Western Ethiopia. Faculty of Veterinary Medicine AAU, Debre Zeit, Ethiopia, DVM thesis.
- Simenew K, Bekana M, Fikre L, Tilahun Z, Wonda M (2011). Major gross reproductive tract abnormalities in female cattle slaughtered at sululta slaughterhouse in Ethiopia. *Glob. Vet.* 6:506-513
- Singleton GH, Dobson H (1995). A Survey of the reason for culling pregnant cows. *Vet. Rec.* 136:162-164.
- Teklu F (1999). Study on gross and histopathological abnormalities of reproductive tract of cows slaughtered at Addis Ababa abattoir. Faculty of Veterinary Medicine AAU, Debre Zeit, Ethiopia, DVM thesis.
- Zerihun Y (2001). A gross morphological study of genital organs form female Zebu cattle at Raya Valley, North Ethiopia. Faculty of Veterinary Medicine AAU, Debre Zeit, Ethiopia, DVM thesis.

Full Length Research Paper

Occurrences and financial significance of bovine cystic echinococcosis in Southern Wollo, Northeastern Ethiopia

Alemu Bizuwork¹, Nigatu Kebede^{2*}, Tariku Tibat¹, Getachew Tilahun² and Tesfu Kassa²

¹School of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

²Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia.

Accepted 16 December, 2012

The study was carried out in Kombolcha ELFORA Industrial Abattoir to assess the current status and economic importance of bovine hydatidosis. Hydatid cyst count, characterization and economic loss assessment were conducted out of a total of 535 cattles slaughtered in Kombolcha ELFORA Industrial Abattoir. 93 (17.4%) animals were found harboring hydatid cysts. Thorough meat inspection in the abattoir revealed that 101 visceral organs were found harboring one or more hydatid cyst. The infection of the lung, liver, spleen, heart and kidney were found to be 50.5, 40.6, 1.98, 4.95 and 1.98%, respectively. From the total of 276 hydatid cysts counted, 135 (49.6%), 47 (17%), 20 (7.24%) and 72 (26%) were found to be small, medium, large and calcified cysts, respectively and 178 (67.9%), 12 (4.3%) and 72 (26%) were sterile, fertile and calcified, respectively. The rate of cyst calcification was higher in the liver than in the lung while fertility rate was higher among the cysts of the lung. The annual financial losses from organ condemnation and carcass weight loss due to bovine hydatidosis at Kombolcha ELFORA Industrial Abattoir were estimated to be 288,473.60 Ethiopian Birr (ETB) (\$1 = 17.00 ETB). Thus, echinococcosis/hydatidosis is considerably a prevalent disease in cattle, with serious public health concern reflections and causes substantial visible and invisible losses in cattle in the study area.

Key words: Bovine hydatidosis, economic loss, Kombolcha ELFORA, meat inspection, prevalence.

INTRODUCTION

Among the many prevalent livestock diseases, parasitosis represent a major drawback to livestock development in the tropics in general and hydatidosis is among the major parasite diseases contributing to low productivity of meat production due to carcass or organ condemnation, in particular (Lemm et al., 1985). Infection, with the metacestode hydatid cyst of *Echinococcus granulosus*, stage of parasite tape worms is recognized as one of the world's major zoonosis affecting both humans and domestic animals (Cringoli et al., 2007).

The definitive host of the parasite, *E. granulosus*, is dogs which harbor adult tape worms and excrete the parasite eggs along with their feces, while livestock and

and human are the main intermediate hosts (Oku et al., 2004) for whom the outcome of infection is the development of hydatid cysts in lung, liver or other organs (Muller, 2001; Budke et al., 2006). The incidence of human hydatidosis and the prevalence of the hydatidosis in domestic animals are the highest in countries where there is a large dog population and high sheep production (Gracey, 1986). The absence of proper meat inspection procedures and the presence of large stray dog population are thought to contribute significantly to the prevalence of the disease in Ethiopia (Kebede et al., 2009a). In Ethiopia, a number of researchers reported high prevalence of hydatidosis in different parts of the country. Fuller and Fuller (1981) documented a hyperendemic focus of hydatid disease in South-western Ethiopia in Dassanetch and Nyangatom people. In abattoirs of various locations, researchers indicated that hydatidosis is widespread in

*Corresponding author. E-mail: nigatukebede@yahoo.com Tel: +251112763091. Fax: +251112755296.

Ethiopia with great economic and public health significance (Jobre et al., 1996; Sissay et al., 2008; Kebede et al., 2009a, b, c, d, Kebede, 2010). However, there is no current information regarding the prevalence and economic significance of hydatidosis in livestock in South Wollo zone, northeastern part of the country. Hence, it would be essential to have information on the status of hydatidosis with regard to its magnitude of occurrence and economic significance of this disease in the region. Therefore, the present study was aimed at (1) determining the magnitude of the hydatidosis in cattle; (2) studying the localization and fertility/sterility rates of hydatid cysts; and (3) estimating the annual economic loss attributed to the condemned carcasses and organs and due to weight loss in Kombolcha ELFORA Industrial Abattoir, Northeastern Ethiopia.

MATERIALS AND METHODS

Study area

This study was conducted at Kombolcha ELFORA Industries Abattoir. The abattoir is privately owned which supplies lean meat to Kombolcha Meat Factory for canning. The abattoir is located at Kombolcha, South Wollo, at an altitude of 1500 to 1840 m above sea level and about 375 km away from Addis Ababa in the northeast direction (DoARD, 2006).

Study animals and sampling

A total of 535 cattle presented for slaughter at Kombolcha ELFORA Industrial Abattoir were examined for hydatid cyst in the period between August 2010 and May 2011. The study was an active abattoir survey which included cattle brought from different livestock markets to Kombolcha ELFORA Industrial Abattoir. The sample size was determined by 95% confidence interval at a desired accuracy level of 5% (Thrusfield, 1995) and with expected prevalence of 28.4% (Asrat, 1996). Using purposive sampling method, the samples were selected from cattle registered for slaughtering following the antemortem inspection.

$$n = \frac{1.96^2 p_{exp} (1-p_{exp})}{d^2}$$

Where n = required sample size; p_{exp} = expected prevalence; d = desired absolute precision.

Study design

Postmortem examination

A total of 535 cattle presented for slaughter at Kombolcha ELFORA Industrial Abattoir were examined for the presence of hydatid cyst following the routine meat inspection procedures. The inspection procedure used during the postmortem examination consisted of two steps, namely primary and secondary examination. Primary examination involved usual inspection and palpation of organs and viscera followed by a secondary examination if evidence of metacestode was found. The secondary examination involved further incision into each organ if single or more hydatid cyst(s) were found. The liver, lungs, heart, spleen, mesentery and

omentum of each animal were examined grossly. Each organ was also incised once or twice with knife. Whenever the cysts were present they were removed, placed in polyethylene bags separately, labeled and taken to the laboratory for further examination.

Identification of cysts was done in the parasitology laboratory of Kombolcha Regional Veterinary Laboratory based on the criteria described by Soulsby (1982). During the study, detailed records of the species, age of the animals, number, size, location and viability of the cyst(s) were made. All animals slaughtered were local zebu breed of cattle at the age of 4 years and above.

Hydatid cyst characterization

The infected organs from each positive animal were collected and recorded. The total number of hydatid cysts was counted and recorded for each infected organ. The size of the diameter of collected hydatid cysts was measured and classified as small (diameter less than 5 cm), medium (diameter between 5 and 10 cm) and large (diameter greater than 10 cm) (Kebede et al., 2009a,c; Oostburg et al., 2000).

Individual hydatid cysts were carefully incised and examined for protoscolices, which resembled white dots on the germinal epithelium; such cysts were characterized as fertile cysts. Fertile cysts were subjected to viability test. A drop of the sediment containing the protoscolices were placed on the microscope glass slide and covered with cover slip and observed for amoeboid like peristaltic movements with 40x objective. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscolices in hydatid fluid on microscope slide with the principle that viable protoscolices should completely or partially exude the dye while the dead ones absorb it (Macpherson et al., 1985). Furthermore, infertile cysts were further classified as sterile or calcified. Sterile hydatid cysts were characterized by their smooth inner lining usually with slightly turbid fluid in their content. Typical calcified cysts produce a gritty-sound heard at incision (Soulsby, 1982).

Financial loss estimation

Direct and indirect losses were the basis for the estimation of the annual economic losses. Direct losses were calculated on the basis of condemned organs, whereas the indirect losses were estimated on the basis of live weight loss caused by hydatidosis (Polydorou, 1981; Torgerson and Dowling, 2001).

The parameters considered for the estimation of financial loss were five percent estimated carcass weight loss due to hydatidosis (Polydorou, 1981), slaughter rates of animals at Kombolcha ELFORA Industrial Abattoir, average carcass weight (dressing percentage) of Ethiopian Zebu cattle breed (126 kg) and the mean retail market price of condemned organs due to hydatidosis such as lung, liver, kidney, spleen. The total financial loss due to hydatidosis was the sum of direct and indirect losses.

Direct and indirect financial losses were calculated on a yearly basis. Average market price of lung, liver, spleen, kidney, heart and a kilogram of beef was found to be 30, 45, 15, 10, 15 and 80 Ethiopian Birr (ETB), respectively. The mean annual numbers of cattle slaughtered during the last one year were 5000. Average number of cattle positive for hydatidosis as it was extrapolated from prevalence findings on ELFORA abattoir were 17.4%.

Direct losses were calculated as follows:

$$DL = (AS \times CLu \times PLu) + (AS \times CLi \times PLi) + (AS \times CSp \times PSp) + (AS \times CKid \times PKid) + (AS \times Chr \times Phr)$$

Where DL = direct losses associated with hydatidosis, AS =

estimated mean annual slaughter; PLu = percent involvement of the lung; CLu = local retail price of a lung; PLi = present involvement of the liver; CLi = local retail price of a liver; PSp = present involvement of the spleen; CSp = local retail price of a spleen; PKid = percent involvement of the kidney; CKid = local retail price of a kidney; PHr = percent involvement of the heart; CHr = local retail price of a heart.

Indirect losses (IL) = 5% NAS × PH × CPB × 126 kg

Where 5% = A reduction of 5% in meat production due to hydatidosis established by Polydorou (1981); NAS = average number of cattle slaughtered annually; PH = prevalence rate of hydatidosis; CPB = current average price of 1 kg of beef at Kombolcha; 126 kg is the dressed average carcass weight of adult Zebu cattle (ILCA, 1979).

Total economic loss (TL)

The total economic loss can be evaluated by considering both DL and IL as follows:

TL = DL + IL

Data analysis

Data collected from antemortem, postmortem and laboratory finding were entered in to MS Excel and statistical packages (SPSS Version 18) were employed to analyze the results.

RESULTS

This study demonstrated that at Kombolcha ELFORA Industrial Abattoir, 93 (17.4%) were found harboring one or more hydrated cyst. The result obtained from postmortem examination indicated that different organs were affected with hydatid cyst.

Single and multiple infection of organs were recorded out of the total 93 cattle harboring hydatid cysts; 87 (93.5%) were found involving only a single organ and the remaining 6 (6.5%) had a multiple organ involvement. Among the different organs affected, lung and liver constituted the highest infection rate (Table 1).

Cyst size

Exceptionally two large cysts were found in the lung and spleen of cattle measuring 18 and 17 cm in diameter and containing about 1.5 and 1.3 L of fluid, respectively. The total cyst count with respect to size in each infected organ for cattle was described in Table 2.

Cyst fertility, viability and sterility

Fertility and sterility of hydatid cyst was described. The viability percentage of protoscolices was higher in the lung (5.9%) than in the other organs while the percentage

of calcified cysts was 36.8%, the highest in the liver (Table 3).

Direct financial loss

Due to cattle hydatidosis, 51 lungs, 41 livers, 5 hearts, 2 kidneys and 2 spleens were condemned during the study period with an economic loss of 1530, 1845, 125, 20 and 30 ETB, respectively (Table 4). This was assessed from the mean retail market price of each organs and the total number of organs condemned during the study period. Annual economic loss on the other hand was estimated considering annual slaughter rate of cattle and prevalence of hydatidosis per organ and was calculated to be 24,423.6 ETB per annum.

Indirect loss

The estimated economic loss from carcass weight loss due to hydatidosis was estimated to be 274,050 ETB. Therefore, the total estimated annual economic loss in cattle at Kombolcha ELFORA Industrial Abattoir due to hydatidosis was 302,023.6 ETB (\$1 = 17.00 ETB).

DISCUSSION

The occurrence of hydatidosis in cattle was found to be 17.4% during the study period in Kombolcha ELFORA Industrial Abattoir. The current finding is almost similar to that reported as 16.85% (Jemere and Butako, 2011) in Wolaita Sodo, 15.4% (Regassa et al., 2009) in Hawassa and 16% (Kebede et al., 2009b) in Wolaita Sodo. In general terms, throughout the world, there had been different magnitude records of hydatidosis in cattle with low medium and high rates of occurrences. High prevalence rates were registered in other areas of the country such as 61% in Assela (Koskei, 1998), 52.69% in Hawassa (Regassa et al., 2010), 34.05% in Bahir Dar (Kebede et al., 2009a), 46.5% in Debre Zeit (Jobre et al., 1996), 48.9% in Debre Markos (Kebede et al., 2009c), 32.1% in Mekelle (Berhe, 2009), and 22% in Tigray (Kebede et al., 2009d).

Factors such as difference in culture, social activity, animal husbandry systems, lack of proper removal of infectious carcass, and attitude to dogs in different regions might have contributed to the variation in prevalence in different areas of a country (Arbabi and Hooshyr, 2006) and strain differences of *E. granulosus* that exists in different geographical location (McManus, 2006). In the current study, hydatid cysts were found predominantly in lung and liver representing 50.5 and 40.6 %, respectively. Literature reveals that hydatid cysts are most commonly found in lungs and liver of ungulates (Hubbert et al., 1975) and it is in agreement with the

Table 1. The total number, relative prevalence and number of cysts harbored in affected organ.

Organ	Infected number of organ	Relative prevalence	Total number of cyst
Lung	51	50.5	135
Liver	41	40.6	125
Heart	5	5	10
Kidney	2	2	4
Spleen	2	2	2
Total	101	-	276

Table 2. Cyst size and counts in relation with organ involvements in infected cattle slaughtered in Kombolcha ELFORA.

Organ	Small	Percentage	Medium	Percentage	Large	Percentage	Total
Lung	70	51.9	30	22.2	15	11	115
Liver	58	46.4	17	13.6	4	3.2	79
Heart	4	40	-	-	1	10	5
Kidney	3	75	-	-	-	-	3
Spleen	2	100	-	-	-	-	2
Total	137	49.6	47	17	20	7.24	204

Table 3. Type of hydatid cyst sterile, fertile and calcified indifferent organs of infected cattle.

Organ	Sterile	Percentage	Fertile	Percentage	Calcified	Percentage	Total
Lung	107	79	8	5.9	20	14.8	135
Liver	61	48.8	4	3.2	46	36.8	125
Heart	5	50	-	-	5	50	10
Kidney	3	75	-	-	1	25	4
Spleen	2	100	-	-	0	0	2
Total	178	67.9	12	4.3	72	26	276

Table 4. Direct economic losses associated with CE in infected cattle in Kombolcha ELFORA industrial abattoir, Northeast Ethiopia.

Organ	No. of organs condemned	Price per organ	Total price ETB
Lung	51	20	1530
Liver	41	15	1845
Heart	5	25	125
Kidney	2	10	20
Spleen	2	15	30
Total	101	61	3,550

findings of Bekele and Butako (2011), Njoroge et al. (2002) and Eckert and Deplazes (2004), which show that the lung and liver are the most common sites of hydatid cyst in domestic animals. This could be justified by the fact that lungs and liver possess greater capillary fields,

which allow these organs to efficiently filter the ingested oncospheres from the blood liver and lungs undergo sequential filtration of blood, liver undergoes primary filtration of blood from portal veins which is followed by pulmonary filtering actions before other organs are

invaded (Eckert and Deplazes, 2004). Only those oncospheres which transfer the blood will reach the systemic circulation and other tissues (Eckert and Deplazes, 2004; Matosain, 1977).

High numbers of small, medium and large size cysts were found in lungs than in the liver, while the liver harbored higher number of calcified cysts. The reason for higher percentage of small, medium and large cysts in the lungs is due to soft structure of the lung, while the higher yield of calcified cysts in liver could be attributed to relatively higher reticuloendothelial cells and abundant connective tissue reaction of the organ. The higher proportion of a small cysts may be due to immunological response of the host which might preclude expansion of cysts life (Torgerson et al., 1998; Lahmar et al., 1999; Larrieu et al., 2001; Torgerson, 2002). In examining the condition of cyst fertility and viability, the findings of 67.9% sterile, 4.3% fertile and 26% calcified were examined. It may be concluded that most of the cysts in cattle were infertile. The variation infertility rate among different species and in different geographical zone could be due to the differences in the strain of *E. granulosus* (McManus, 2006). Most of the hydatid cysts from cattle are considered to be sterile (Thompson et al., 1984).

In comparison of the fertility rate among the organs, it was higher in lungs than in liver. It has been stated that the relatively softer consistency of the lung tissue allows easier development of the cysts and the fertility rate of hydatid cysts may show a tendency to increase with advancing the age of the hosts (Himonas et al., 1987). This may be attributed to reduced immunological compatibility of animals at their older age of infection. The variation between tissue resistances of the infected organs may also influence the fertility rate of hydatid cysts. The fertility rates observed in this study are low; however, could serve as potential source to infection and perpetuate the cycle of hydatidosis when infected animals are slaughtered and raw offal fed to dogs and also leftovers during backyard slaughter are eaten by wild carnivores. It was observed that majority of the households had livestock, including cattle, sheep, goat, and donkeys, which are the intermediate host of the parasite. Similarly, many households had dogs and cats, which were not dewormed regularly and were managed under free-range system. In this study, hydatidosis was found to incur financial loss that is estimated to be 302,023.60 ETB to the cattle industry per year. Previous studies have also estimated the annual financial losses associated with bovine hydatidosis from other parts of the country. For instance, Bekele and Butamo (2011) reported 410,755.90 ETB (30,202.64 US\$), Kebede et al. (2009b) reported 25,608 ETB, Regassa et al. (2010) reported 1,791,625.89 ETB. The difference in the calculated economic loss in the various abattoirs is either due to the variation in the number of slaughtered animals or variation in the prevalence rate of hydatidosis or due to variation in the retail market price of organs. Based on our results, we suggest that bovine hydatidosis is among

many of the livestock diseases prevailing in the country incurring both direct and indirect losses to the cattle industry due to condemnation of organs and reduced live weight gain of infected cattle.

In Kombolcha ELFOR Industrial Abattoir, condemned organs and carcasses are buried and some of them are further processed for animal feed. This may reduce the contamination and infection in dogs and other carnivores. Therefore, the decreased prevalence of hydatidosis in this study as compared to the previous studies conducted in different areas of the country could be due to proper disposal of the condemned organ where dogs have no access to infected organs. However, there are backyard slaughter practices during local festivities, the tradition of offering uncooked infected offals to pet animals around homestead, poor public awareness about the diseases, and the habit of disposing dead wild or domestic animals unburied and left open for scavenging carnivores creates favorable condition for maintaining the life cycle of *E. granulosus* in stray dogs and wild carnivores in the area.

Conclusion

This study highlights the need for thorough investigation to obtain appropriate and more accurate information on the incidence of hydatidosis/echinococcosis in humans, dogs, both in domestic and wild animals to determine the scope and type of relevant control options. In spite of the low magnitude of infection detected, there seems to be an existing socioeconomic condition favorable for hydatidosis, and hence, it remains one of the most important diseases warranting serious attention for prevention and control actions.

ACKNOWLEDGEMENTS

The authors acknowledged Kombolcha Regional Veterinary Laboratory and administrative and technical staffs of Kombolcha ELFOR Industrial Abattoir for their cooperation during the study. Financial support was obtained from Addis Ababa University.

REFERENCES

- Arbabi M, Hooshyr H (2006). Survey of echinococcosis and hydatidosis in Kashan region central Iran. Iran. J. Pub. Health 35(1):75-81.
- Bekele J, Butako B (2011). Occurrence and financial loss assessment of cystic echinococcosis (hydatidosis) in cattle slaughtered at Wolayita Sodo municipal abattoir, Southern Ethiopia. Trop. Anim. Health Prod. 43(1):221-228.
- Berhe G (2009). Abattoir survey on cattle hydatidosis in Tigray region of Ethiopia. Trop. Anim. Health Prod. 41(7):1347-1352.
- Budke CM, Deplazes P, Torgerson PR (2006). Global socio economic impact of cystic echinococcosis. Emerg. Infect. Dis. 12(2):296-303.
- Cringoli G, Rinaldi L, Musella V, Veneziano V, Maurelli MP, Di Pietro F, Frisiello M, Di Pietro S (2007). Geo-referencing livestock farms as tool for studying cystic echinococcosis epidemiology in cattle and water buffaloes from southern Italy. Geospat. Health 2(1):105-111.

- Department of Agriculture and Rural Development for South Wollo Zone (DoARD) (2006). Basic data of North Wollo Zone Agricultural Department. pp. 1-25.
- Eckert J, Deplazes P (2004). Biological, epidemiological and clinical aspects of echinococcosis a zoonosis of increasing concern. *Clin. Microbiol. Rev.* 17(1):107-135.
- Fuller GK, Fuller DC (1981). Hydatid disease in Ethiopia: Clinical survey with some immunodiagnostic test results. *Am. J. Trop. Med. Hyg.* 30(3):645-652.
- Gracey JE (1986). *Meat hygiene 8th Ed.*, Bailliera Tindall, London pp 100-101.
- Himonas C, Frydas S, Antoniadou-Sotiriadou K (1987). The fertility of hydatid cysts in food animals in Greece. In: Geerts S, Kumar V, Brandt J Eds. *Helminth zoonosis* Martinus, Nijjih of publishers, Netherlands. pp 12-21.
- Hubbert WT, Culloch WF, Schnurren Beger AA (1975). *Disease Transmitted from animal to man.* 6th Ed., Choleler C. Thomas Publisher, Spring Filk Illinois.
- ILCA (1979). Trypanotolerant livestock in West and central Africa: General study. ILCA Monograph 2, ILCA, Addis Ababa, Ethiopia 1:147.
- Jobre Y, Labago F, Tiruneh R, Abebe G, Dorchie PH (1996). Hydatidosis in three selected regions of Ethiopia: An assessment trail on the prevalence, economic and public health importance. *Rev. Med. Vet.* 11(147):797-804.
- Kebede N (2010). A retrospective survey of bovine hydatidosis in three abattoirs of Amhara National Regional State, northwestern Ethiopia. *Trop. Anim. Health Prod.* 42(3):323-325.
- Kebede N, Mitiku A, Tilahun G (2009a). Hydatidosis of slaughtered animals in Bahir Dar Abattoir, Northwestern Ethiopia. *Trop. Anim. Health Prod.* 41(1):43-50.
- Kebede N, Mokonnen H, Wossene A, Tilahun G, (2009b). Hydatidosis of slaughtered cattle in Wolayita Sodo abattoir southern Ethiopia. *Trop. Anim. Health Prod.* 41:629-633.
- Kebede N, Abuhay A, Tilahun G, Wossene A (2009c). Financial loss estimation, prevalence and characterization of hydatidosis of cattle slaughtered at Debre Markos municipality abattoir, Ethiopia. *Trop. Anim. Health Prod.* 41:1787-1789.
- Kebede W, Hagos A, Girma Z, Labago F (2009d). Echinococcosis/hydatidosis: Its prevalence, economic and public health significance in Tigray region, North Ethiopia. *Trop. Anim. Health Prod.* 41(6):865-871.
- Koskei PK (1998). Prevalence and strain differentiation of *Echinococcus granulosus* in some selected sites of Ethiopia. Berlin and Ethiopia: Ferie Universitat and Addis Ababa University, M.Sc. thesis.
- Lahmar S, Kilani M, Torgerson PR, Gemmel M A (1999). *Echinococcus granulosus* larvae in the livers of sheep in Tunisia: The effects of host age. *Ann. Trop. Med. Parasitol.* 93(1):75-81.
- Larriue E, Costa T, Cantoni G, Alvarez R, Cavagion L, Labanchi JL, Bigatti R, Araya D, Herrero E, Alvarez E, Mancini S, Cabrera P (2001). Ovine *Echinococcus granulosus* transmission dynamics in the province of Rio Negro, Argentina, 1980-1999. *Vet. Parasitol.* 98(4):263-72.
- Lemm B, Gabre F, Tedla S (1985). Studies on fascioliasis in four selected sites in Ethiopia. *Vet. Parasitol.* 18(1):29-37.
- Macpherson CNL, Zeyhle E, Roming T (1985). An echinococcosis pilot control programme for Northwest Turkan, Kenya. *Ann. Trop. Med. Parasitol.* 78(3):188-192.
- Matosain R M (1977). Hydatidosis: A global problem of increasing importance. *Bull World Health Organ.* 55(4):499-507.
- McManus DP (2006). Molecular discrimination of taeniid cestodes. *Parasitol. Int.* 55:S31-37.
- Muller R (2001). *Worms and Human Disease.* CAB International, Oxon, UK. pp 85-86.
- Njoroge EM, Mbithi PM, Gathuma JM, Wachira TM, Magambo JK, Gathura PB, Zeyhle E (2002). A study of cystic echinococcosis in slaughter animals in three selected areas of north Turkana, Kenya. *Vet. Parasitol.* 104(1):85-91.
- Oku Y, Malgorb R, Benavidez U, Crmonab C, Kamiyac H (2004). Control program against hydatidosis and the decreased prevalence in Uruguay. *Int. Congr. Ser.* 1267:98-104.
- Oostburg BFJ, Vrede MA, Bergen AE (2000). The occurrence of polycystic echinococcosis in Suriname. *Ann. Trop. Med. Parasitol.* 94(3):247-252.
- Polydorou K (1981). Animal health and economics case study: Echinococcosis with reference to Cyprus. *Bull. Int. Epizoot.* 93(5):981-992.
- Regassa A, Abunna F, Mulugeta A, Megersa B (2009). Major metacestodes in cattle slaughtered at Wolayita Sodo municipal abattoir, Southern Ethiopia: Prevalence, cyst viability, organ distribution and socioeconomic implications. *Trop. Anim. Health Prod.* 41(7):1495-15502.
- Regassa F, Molla A, Bekele J (2010). Study on the prevalence of cystic hydatidosis and its economic significance in cattle slaughtered at Hawassa municipal abattoir. *Trop. Anim. Health Prod.* 42(5):977-984.
- Sissay MM, Ugglala A, Waller PJ (2008). Prevalence and seasonal incidence of larval and adult cestode infections of sheep and goats in eastern Ethiopia. *Trop. Anim. Health Prod.* 40(6):387-394.
- Soulsby E JL (1982). *Helminthes, Arthropods and Protozoa of domesticated animals 7th Ed.* English Language book society/Billiere Tindall, London pp 123-127.
- Thompson RCA, Kumaratilke LM, Eckert J (1984). Observations on *Echinococcus granulosus* of cattle origin in Switzerland. *Int. J. Parasitol.* 14(3):283-291
- Thrusfield M (1995). *Veterinary Epidemiology, 2nd Ed.* Edinburgh, Black well science Ltd., UK. pp 182-198.
- Torgerson PR, Dowling PM (2001). Estimating the economic effects of cystic echinococcosis. Part 2: An endemic region in the United Kingdom, a wealthy, industrialized economy. *Ann. Trop. Med. Parasitol.* 95(2):177-85.
- Torgerson P (2002). Transmission dynamics of taeniid parasites in animal hosts. In: P. Craig and Z. Pawlowski (Ed.), *Cestode zoonoses: Echinococcosis and cysticercosis, an emergent and global problem.* The Netherlands, Amsterdam IOS press pp 221-235.
- Torgerson PR, Williams DH, Abo-Shehada MN (1998). Modelling the prevalence of *Echinococcus* and *Taenia* species in small ruminants of different ages in northern Jordan. *Vet. Parasitol.* 79(1):35-51.

Full Length Research Paper

Molecular detection of canine parvovirus in Jos, Nigeria

**S. C. Chollom^{1,2}, E. J. Fyaktu², A. E. J. Okwori³, G. O. A. Agada⁴, G. Hashimu⁵, R. Y. Akele³,
E. I. Voumangai⁶, T. Dashe⁷ and D. Z. Egah³**

¹Viral Research Department, National Veterinary Research Institute, Vom, Nigeria.

²Department of Medical Microbiology, University of Jos, Nigeria.

³Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute, Vom, Nigeria.

⁴Central Diagnostics Laboratory, National Veterinary Research Institute, Vom, Nigeria.

⁵Bacterial Vaccine Production Department, National Veterinary Research Institute, Vom Nigeria.

⁶Laboratory Department, Plateau State Specialist Hospital, Jos, Nigeria.

⁷Institute of Human Virology Nigeria, Plateau State Virology Research Centre, Plateau State Specialist Hospital Jos, Nigeria.

Accepted 28 November, 2012

Canine parvovirus (CPV) remains the most significant viral cause of enteritis in puppies over the age of two months. This study was meant to detect the virus by targeting the VP2 gene, a 583 bp gene (nucleotide 4003 to 4585) of the capsid protein. The detection of the virus was carried out by conventional polymerase chain reaction (PCR) on one hundred and nine samples. Seventy five of these were rectal swabs while thirty four were necropsy tissues, all from dogs presenting with symptoms suggestive of parvovirus enteritis from veterinary clinics in Jos metropolis. Results revealed that 47.70% of the samples were found to be positive for the virus. The rate of detection was more in necropsy tissues (64.71%) relative to rectal swabs (40.00%). A breakdown of the incidence of the infection across breeds of dogs showed that the Rottweillers had 60.00%, Doberman pinchers had 54.54%, Pitbulls had 53.33% while Tan coloured and local breeds of dogs had 50.00 and 21.42%, respectively. With a prevalence rate of 47.70%, the findings have confirmed that the virus is circulating in Jos, Nigeria. As such, stake holders must quickly intervene to arrest the situation given the high economic losses associated with the disease.

Key words: Canine parvovirus, polymerase chain reaction (PCR), Jos.

INTRODUCTION

Canine parvovirus (CPV) is a highly contagious virus mainly affecting dogs. The disease, commonly called parvovirus enteritis is highly infectious and is transmitted from dog to dog by direct or indirect contact with contaminated feces (Appel et al., 1979). Parvovirus enteritis is a relatively new disease that appeared in the late 1970s. It is highly contagious and often fatal in both domestic and wild dog populations with high morbidity (100%) and frequent mortality of up to 10% (Appel, 1978). It is charac-

terized by vomiting, bloody diarrhea, myocarditis and leucopenia (Streck et al., 2009). CPV was first recognized in 1978 and spread worldwide in one to two years (Carmichael, 2005). The virus is a small, non enveloped single stranded DNA virus belonging to the Parvoviridae family and parvovirus genus under the Parvovirinae sub-family (Hong et al., 2007). The virus, which first appeared between 1977 to 1978, probably arose from a very closely related virus in cats, feline panleukopaenia virus (FPLV) through a small number of mutations in the single capsid protein; a species jump which may have involved intermediate passage in other carnivores such as mink or raccoons (Truyen et al., 1996). As early as 1979 the first

*Corresponding author. E-mail: cholloms_12@yahoo.com. Tel: 2348036574286.

Table 1. Percentage prevalence according to sample type.

Sample type	PCR positive (%)
Swabs (n = 75)	30 (40.00)
Tissues (n = 34)	22 (64.71)
Total (T = 109)	52 (47.70)

Table 2. Percentage positivity of samples based on breed of dog.

Breed	PCR positive (%)
Rottweillers (n = 25)	15 (60.00)
Doberman Pinchers (n = 33)	18 (54.54)
Pit Bulls (n = 15)	08 (53.33)
Tan Coloured (n = 04)	02 (50.00)
Local (n = 28)	06 (21.42)
Unidentified (n = 04)	03 (75.00)
Total (T = 109)	52 (47.70)

variants of CPV2 appeared, termed CPV2a, and they were quickly followed by the appearance of CPV2b in 1984 (Parrish et al., 1985, 1991). The original type 2 virus has now disappeared from the field having been replaced by the 2a and 2b variants; although the relative proportions of these two types varies from country to country (Truyen et al., 1996; Chinchkar et al., 2006; Pereira et al., 2007). However, in Africa, including Nigeria, no reports as regarding the circulation of the virus in the area is available (Kapil et al., 2007). This study is aimed at detecting the virus using polymerase chain reaction (PCR) in Jos, Nigeria.

MATERIALS AND METHODS

A total of one hundred and nine samples were collected. Seventy five of which were rectal swabs while thirty four were necropsy tissues. The rectal swabs were collected from dogs presenting with bloody diarrhea and vomiting signs while necropsy tissues of target organs (intestine, heart, lymph nodes, spleen and liver) were harvested from dogs that died from the disease.

Sample collection

Rectal swabs were collected by the insertion of the cotton bud of a swab stick (Steriline®) through the anal region of the animal into the rectum after the dog has been restrained. The swab stick was carefully turned clockwise and gently withdrawn. A scissors was used to cut off the swab bud into 2 ml cryovial containing virus transport medium (VTM) and transported to the laboratory in a cold box. Necropsy tissues of target organs were collected from dead dogs. They were aseptically delivered into a universal sample bottle (Steriline®) and transported to the laboratory in a cold box.

Polymerase chain reaction (PCR)

Total DNA was extracted using the QIAamp® DNA Mini Kit according to manufacturer's specifications. Different procedures were adopted for DNA extraction from swabs and DNA extraction from tissues.

CPV-2 specific master mix

The master mix contained 13.88 µl of nuclease free water (Promega®), 2.5 µl of 10× PCR reaction buffer, 0.62 µl of dNTP mixture (10 mM) (Fermentas®), 1.5 µl of magnesium chloride (25 nM), 0.5 µl of forward and 0.5 reverse primers (555 forward and 555 reverse, respectively) for CPV2 (Inqaba Biotech SA®) at 20 pmol/µl and 0.5 units of Taq DNA polymerase (Qiagen®). This gave a total volume of 20 µl master mix. 5 µl of the CPV-2 master mix (above) was added to 5 µl extracted DNA in a new tube for amplification.

Amplification of DNA extracts

The mixture was submitted to a thermal cycling profile of initial denaturation at 94°C for 5 min. This was followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 2 min, extension at 72°C for 2 min and 72°C for 10 min in an Applied Biosystem® 9700 PCR machine. The primer sequences used were as follows: 555 forward, 5'-CAGGAAGATATCCAGAAGGA-3' and 555 reverse, 5'-GGTGCTAGTTGATATGTAATAAACA-3'. The master mix composition and PCR program was as described by Streck et al. (2009) with minor modifications.

Electrophoresis and analysis

A 10 µl of PCR amplicons was electrophoresed in a 1.5% agarose gel stained with ethidium bromide in the presence of 2 µl of gel loading buffer (Fermentas®). Positive and negative controls were included. The electrophoresis was carried out at 80 volts for 50 min.

RESULTS

Amplicon band sizes of about 583 bp were considered positive for the virus. Results revealed that 52 (47.70%) of the one hundred and nine samples were positive for the virus.

From the seventy five rectal swabs examined, 30 (40.00%) of them were positive for CPV just as 22 (64.71%) out of the thirty four tissue samples examined and were positive for the virus by conventional PCR (Table 1). Statistically, there was no significant difference ($P > 0.05$) in the number of positive samples from the sample types surveyed (Table 1). Although the sample size was small for breed comparison, Rottweillers had 60.00% incidence, Doberman pinchers 54.54%, Pitbulls 53.33%, Tan coloured 50.00% while local breeds of dogs had only 21.40% (Table 2).

DISCUSSION

Canine parvovirus continues to be an important pathogen

of dogs and it is responsible for serious occurrences of morbidity and mortality despite the availability of safe and effective vaccines (Decaro et al., 2006a,b). This study revealed that 52 out of the 109 samples examined had the virus. This study therefore revealed a prevalence rate of 47.70% of the disease from this part of the world. The figure is less compared with seven positive samples of CPV 2c strain obtained from nine samples (77.78%) assayed in Brazil (Streck et al., 2009). Also, it is lower than the findings in Uruguay where 24 out of 25 faecal samples (96.00%) were found to be positive for one strain of the virus (Perez et al., 2007). The high percentage of positive tissues against rectal swabs is not unconnected with the fact that the selected tissues had earlier been established as the predilection sites of the agent as such, it is only natural that the virus concentration in such sites be higher (Lobetti, 2003).

Local breeds of dogs were the least susceptible to the infection as against their foreign counterparts. Authors had previously suggested that the former have a greater degree of resistance against the virus than the foreign breeds. They have also been fingered as healthy carriers of the virus (Nelson and Couto, 1998). This is of great epidemiological relevance as their role in the distribution of the virus is once again re-echoed. Since most of the local breeds are free rangers, it makes it possible for them to distribute the virus indiscriminately. This factor poses a great danger to the foreign breed of dogs which are more susceptible to the agent as their local counterparts (Nelson and Couto, 1998).

Although canine parvovirus was implicated in 52 (47.70%) of the samples as causative agent responsible for enteritis in these dogs, the cause of enteritis in 57 (52.29%) PCR-negative samples may be due to other pathogens such as *Leptospira* or *Eimeria* species. In general, these findings will no doubt interest both the local and international communities as they have waited all this while for a report on the agent from this part of the world (Kapil et al., 2007).

REFERENCES

- Appel MJG, Cooper BJ, Greisen H, Carmichael LE (1978). Status report: Canine viral enteritis. *J. Am. Vet. Med. Assoc.* 173:1516-1518.
- Appel MJG, Scott WF, Carmichael LE (1979). Isolation and immunization studies of canine parvo-like virus from dogs with haemorrhagic enteritis. *Vet. Rec.* 105(8):156-159.
- Carmichael L (2005). An annotated historical account of canine parvovirus. *J. Vet. Med. Infect. Dis. Vet. Public Health* 52(7-8):303-311.
- Chinchkar SR, Mohana SB, Hanumantha RN, Rangarajan PN, Thiagarajan D, Srinivasan, VA (2006). Analysis of VP2 gene sequences of canine parvovirus isolates in India. *Arch. Virol.* 151(9): 1881-1887.
- Decaro N, Desario C, Elia G, Campolo M, Lorusso E, Mari V, Martella V, Buonavoglia C (2006a). Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: Clinical laboratory diagnostic dilemma. *Vaccine* 25(7):1161-1166.
- Decaro N, Martella V, Desario C, Bellacicco AL, Camero M, Manna L, d'Aloja D, Buonavoglia C (2006b). First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. *J. Vet. Med. Infect. Dis. Vet. Public Health* 53:468-472.
- Kapil S, Cooper E, Lamm C, Murray B, Rezabek G, Johnston L, Campbell G, Johnson B (2007). Canine parvovirus types 2c and 2b circulating in North American Dogs in 2006 and 2007. *J. Clin. Microbiol.* pp. 4044-4047.
- Lobetti R (2003). "Canine Parvovirus and Distemper". Proceedings of the 28th World Congress of the World Small Animal Veterinary Association. <http://www.vin.com/proceedings/Proceedings.plx>
- Nelson RW, Couto CG, (1998). *Small Animal Internal Medicine* (2nd ed.) Mosby Publisher.
- Pereira CAD, Leal ES, Durigon EL (2007). Selective regimen shift and demographic growth increase associated with the emergence of high fitness variants of canine parvovirus. *Infect. Genet. Evol.* 7 (3):399-409.
- Perez R, Francia L, Romeo V, Maya L, Lopez I, Hernandez M (2007). First detection of canine parvovirus type 2c in South America. *Vet. Microbiol.* 124:147-152.
- Streck AF, de Souza CK, Goncalves KR, Zang L, Pinto LD, Canal CW (2009). First detection of canine parvovirus type 2c in Brazil. *Braz. J. Microbiol.* 40(3).
- Truyen U, Evermann JF, Vieler E, Parrish CR, (1996). Evolution of canine parvovirus involved loss and gain of feline host range. *Virology* 215: 186-189.

UPCOMING CONFERENCES

11th International Congress on the Biology of Fish, Edinburgh, Scotland, 3 Aug 2014



International Conference on Coelenterate Biology, Eilat, Israel, 1 Dec 2013



Conferences and Advert

October 2013

11th World Conference on Animal Production, Beijing, China, 15 Oct 2013

September 2013

International Conference on Optimizing Productivity of Ruminants,
Poultry, Rabbits and Fishes, Marsa Alam, Egypt, 2 Sep 2013

Journal of Veterinary Medicine and Animal Health

Related Journals Published by Academic Journals

- *Journal of Parasitology and Vector Biology*
- *Journal of Cell Biology and Genetics*
- *Journal of Infectious Diseases and Immunity*
- *Journal of Public Health and Epidemiology
Medical Case Studies*
- *Journal of Medical Laboratory and Diagnosis*
- *Journal of Clinical Virology Research* ■ *Medical
Case Studies*

academicJournals