Journal of Veterinary Medicine and Animal Health

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.

ARTICLES

Research Articles

Full Length Research Paper

Effects of three Chinese herbal crude polysaccharides on immunoglobulin A secreting cells and serum antibody titers in vaccinated chickens

Yan QIU[1]*, Yuan-Liang HU[2], Fa-Ming DONG[1], De-Yun WANG[2] and Zhan-Qin ZHAO[1]

[1]College of Animal Technology, Henan Science and Technology University, Luoyang, 471003, P. R. China.
[2]College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, 210095, P. R. China.

This study was conducted to evaluate the effects of three Chinese herbal crude polysaccharides on immunoglobulin A secreting cells and serum antibody titers in vaccinated chickens. A total of 450 14-day-old chickens were randomly assigned to nine equal groups and all chickens were vaccinated. Concurrent with the first vaccination, chickens in groups 1 to 8 were intramuscularly injected with four crude polysaccharides among which the astragalus polysaccharide (APS) was selected as positive control at high and low doses, and group 9 (control group) with saline once a day for three successive days. The numbers of positive immunoglobulin A (IgA) secreting cells and the serum specific immunoglobulin G (IgG) antibody were determined by immunohistochemistry and micro method. The results showed that the individual administration of any of the three crude polysaccharides could significantly increase the number of IgA secreting cells, and the maximum numbers of increased IgA secreting cells in the cecum tonsil and duodenum in the three polysaccharides groups were 37.7 and 33.5 when compared with the controls, and those of the APS groups were 33.9 and 32.7. These three crude polysaccharides at appropriate doses also significantly enhance anti-Newcastle disease virus antibody titers, and the maximum antibody titer increase in the three polysaccharides groups was 1.6 $\log_2$ when compared with the control group, and those of the APS groups was 1.7 $\log_2$. These findings indicated that the appropriate doses of the three crude polysaccharides possess significant immune enhancing properties of mucosa and humoral immune responses, which have similar effect with astragalus polysaccharide, and may be useful as a new type of immune potentiator during vaccination.

Key words: Chinese herbal crude polysaccharides, vaccine, immunity, chickens.

INTRODUCTION

In recent years, many unknown and latent forms of infections have emerged in addition to the prevailing diseases. Among the various emerging diseases, viral

*Corresponding author. E-mail: qiuyan800429@yahoo.com.cn. Tel/Fax: +86 379 64282341.

Abbreviations: APS, Astragalus polysaccharide; **IRPS,** isatis root polysaccharide; **ARPS,** achyranthes root polysaccharide; **CYPS,** Chinese yam polysaccharide; **ND,** Newcastle disease; **IB,** infectious bronchitis; **HI,** hemagglutination inhibition; **IgG,** immunoglobulin G; **IgA,** immunoglobulin A; **CMF,** calcium and magnesium-free; **PBS,** phosphate-buffered saline; **DAB,** diaminobenzidine.

diseases in general and immunosuppressive viruses in particular are suspected as the etiological agents for a variety of clinical conditions in poultry. Newcastle disease (ND) virus is one of the most important avian infection agents, because of high mortality rates in acute infections caused by subclinical infections. Many researchers in Africa, Asia and Australia have identified ND as the major cause of mortality in chicken production. To protect chickens against ND virus, both live and inactivated vaccines have been used. The goal of vaccination is to generate a strong immune response providing long term protection against infection. However, it is difficult to provide full protection of chickens against virus infection. Thus, it is necessary to study and develop safer and more efficacious vaccine immune potentiators that are safe for

chickens and have no risk of producing antigenic and pathogenic variants. The simultaneous application of a vaccine and an immune potentiator could improve the efficacy of vaccination.

Many Chinese herbal medicines and their ingredients have been reported to enhance immune responses (Hu, 1997; Xie, 2000; Ma et al., 2012), and thus have great potential in practical applications. Polysaccharides, one of the main classes of bioactive substances from Chinese herbal medicine, have been indicated to have wide pharmacological activities, especially broad immunomodulatory and antitumour effects. Thus, polysaccharides are regarded as biological response modifiers and attract attention, because of their natural origin, low toxicity in humans and animals, and long-standing use as folk medicines (Lu et al., 2003; Yon et al., 2006). It has been reported that astragalus, isatis root, achyranthes root and Chinese yam are common traditional Chinese medicinal plant widely used to enhance the body's natural defense mechanisms and the immune responses, and polysaccharides are the main effective components of immunological enhancement from these Chinese herbal medicines. Especially, the immune enhancement of astragalus polysaccharide has been reported by many researchers, and has been successfully used in livestock breeding industry (Cho et al., 2007; Cui et al., 2011; Sun and Xie, 2011; Zhao et al., 2008). In this study, we investigated the immunoenhancing effects of three kinds of crude polysaccharides with respect to mucosal and humoral immune responses following vaccination in chickens, astragalus polysaccharide as positive control. The aim of this study is to determine the potential of these three polysaccharides as a new type immune potentiator during vaccination.

MATERIALS AND METHODS

Preparation of Chinese herbal crude polysaccharides

The four crude polysaccharides were extracted using water-decoction-ethanol-precipitation method as previously described (Xue, 1985). Total polysaccharide was measured by Vitriol-anthracene ketone, using glucose without H_2O as a standard control (Liu et al., 1994). The contents (%) of total astragalus polysaccharide (APS), isatis root polysaccharide (IRPS), achyranthes root polysaccharide (ARPS) and Chinese yam polysaccharide (CYPS) (comparable with those of glucose) were 65.1, 56.4, 54.3 and 72.8%, respectively. Based on our previous studies and on the polysaccharide content of the extracts prepared here, the four crude polysaccharides were diluted with deionized water into 2 concentrations as follows: 4 and 2 mg/ml for APS, 3 and 1.5 mg/ml for IRPS, 6 and 3 mg/ml for ARPS and CYPS, respectively. The diluted preparations were sterilized by pasteurization and tested for endotoxin by pyrogen tests (Veterinary Pharmacopoeia Commission of the People's Republic of China, 2000). Following confirmation that all polysaccharide crude extracts met the acceptable standard of Chinese Veterinary Pharmacopoeia (less than 0.5 EU/ml), the preparations were stored at 4°C until use.

Reagents

Mouse anti-chicken IgA antibody (dilution: 1:100), biotinylated rabbit anti-mouse immunoglobulin (IgG) antibody, horseradish peroxidase-labeled chain ovalbumin (all from Shenzhen Jingmei Biotechnology Co. Ltd., Shenzhen, China), normal goat serum (Henan Tumor Hospital Pathology Laboratories, Zhengzhou, China), developer diaminobenzidine (DAB, Sigma) purchased from Beijing Zhongshan Biotechnology Co. Ltd., Beijing, China; hydrogen peroxide, citric acid, sodium citrate are produced by Zhejiang Mitaka chemical reagent Co., Ltd., Lanxi, China; methanol, formaldehyde are produced by Hubei Xinda Li Biochemical Co., Ltd., Wuhan, China; sodium dihydrogen phosphate, disodium hydrogen phosphate are produced by Bei Jing Kang Pu Hui Wei Technology Co., Ltd., Beijing, China.

Vaccine

ND (Lasota strain)-IB (H_{120} strain) live virus vaccine (No. 315) and ND-IB oil adjuvant vaccine (No. 551) were provided by the Institute of Veterinary Medicine, Animal Husbandry Bureau of Henan Province, Zhengzhou, China.

Birds and housing

One-day-old white Roman male chickens (layer type), purchased from Zhengzhou Ruixiang Co. Ltd., were housed in wire cages (60 × 60 × 100 cm) in climate controlled rooms at 36±1°C and kept under 24 h light at the beginning of the pretrial period, with ten chickens per wire cage. The temperature was gradually reduced to room temperature in spring and the light time was kept constant to 12 h per day. Chickens were fed with a commercial starter diet, provided by Feed Factory of Animal Husbandry Bureau of Henan province.

Experimental design

At 14 days of age, 450 chickens were vaccinated with ND-IB live virus vaccine by intranasal and intraocular administrations, and then were randomly divided into nine treatment groups of 50 chickens each, 5 cages per treatment. Their mean titer of maternal antibody against ND virus was 4.5 $\log_2$ and the average body weight was 97.6 g. Each chicken in groups 1 to 8 was injected subcutaneously with 0.5 ml of one of the four crude polysaccharides at one of two concentrations, once a day for three successive days. In group 9, as the control, each chicken was injected with 0.5 ml saline at the same times as treatment groups. At 28 days of age, all chickens were vaccinated for the second time with ND-IB oil adjuvant vaccine by subcutaneous injection in the dorsal region of the cervix. On days 10, 20, 30, 40, 50, and 60 after the first vaccination, eight chickens were sampled randomly from each group to determine changes in the number of IgA secreting cells in the duodenum and cecum tonsil mucosa by immunohistochemistry (Yang et al., 2002), and to determine temporal changes of serum ND antibody titers by micro method (Thekisoe et al., 2004).

Immunohistochemical examination for IgA secreting cells

After sacrifice, a fragment of the duodenum from the same region and cecum tonsil from the same side of each chicken were excised, fixed in 10% neutral formalin solution and embedded in paraffin. Immunohistochemistry was performed on 0.6 mm thick paraffin sections. After deparaffinization and dehydration, endogenous peroxidase was inactivated by incubation with 0.3% hydrogen peroxide in methanol, and was washed in phosphate-buffered saline

(PBS, 0.01 M phosphate, 0.13 M NaCl, pH 7.4) for 10 min, then demasked by microwave oven treatment and citrate buffer. After washing in PBS, the sections were treated with 5% normal goat serum in PBS in a humid chamber for 30 min at room temperature to block non-specific binding. The sections were rinsed three times with PBS for 5 min and then stained separately with monoclonal mouse anti-chicken IgA antibody and the preparations were incubated at 4°C overnight. Tissues were rinsed in PBS for 5 min and then incubated for 30 min with biotinylated secondary antibody diluted (1:50) in PBS. After rinsing with PBS, sections were incubated with horseradish peroxidase-labeled chain ovalbumin for 30 min, washed with PBS and the reactions were made visible with DAB substrate. Sections were then counterstained with hematoxylin, rinsed with distilled water and cleared with xylene. All incubations were performed in a moist chamber. Control staining was carried out simultaneously, in which the primary antibody was replaced with PBS.

Hemagglutination inhibition examination for serum specific IgG antibody

Blood samples (1.0 ml per chicken) were drawn into Eppendorf tubes from the main brachial vein of the chicken and allowed to clot at 37°C for 2 h prior to collecting serum. Serum was separated by centrifugation and stored at -20°C for use. Briefly, after inactivation of serum at 56°C for 30 min, two-fold serial dilution of serum in a 96-well, V-shaped bottom microtiter plate containing 50 μl PBS was performed, and 50 μl of ND virus antigen (4 hemagglutinin (HA) units) was added to all the wells except for the last row, as controls. Serum dilutions ranged from 1:2 to 1:2048. The antigen serum mixture was incubated for 10 min at 37°C. Then 50 μl of a 1% rooster erythrocyte suspension was added to each well and re-incubated for 30 min. Positive serum, negative serum, erythrocytes, and antigens were included as controls. The highest dilution of serum causing complete inhibition of erythrocyte agglutination was considered the endpoint. The geometric mean titer was expressed as reciprocal $\log_2$ values of the highest dilution that displayed anti-ND virus hemagglutination inhibition.

Statistical analysis

The sections were observed using an LEICA microscope (Model DM2000, Germany, purchased from Leica Microsystems Trading Ltd. Shangha, China) and analyzed by Qwin image analysis system of LEICA image workstations (CD 2000, Germany, purchased from Leica Microsystems Trading Ltd., Shangha, China). Twenty different fields of view were chosen per section, with 8 sections per group and analysis of positive IgA secreting cells which appear as brown (Figure 1) was calculated by number. The number of IgA secreting cells in regional units was used for the statistical analysis of the data.

Data are expressed as mean ± standard deviation for analysis; single factor analysis of variance (ANOVA) test was performed using Statistical Package for Social Sciences (SPSS) to determine the difference among herbal polysaccharides and control groups. $P < 0.05$ was considered significant for all analyses.

RESULTS

Increased numbers of IgA secreting cells in duodenum of treated chickens

On day 20 after the first vaccination, the numbers of IgA secreting cells in the duodenum in the APS$_L$ group were significantly elevated when compared with controls   (P <

0.05). On day 30, the numbers of IgA secreting cells in APS$_H$, APS$_L$, IRPS$_L$, ARPS$_H$ and CYPS$_H$ groups were significantly elevated when compared with the controls (P< 0.05), and the largest mean number of IgA secreting cells was 139.1 in IRPS$_L$ group, and 133.2 in APS$_H$, which in control group was 101.4. On days 40 and 50, the numbers of IgA secreting cells in APS$_L$, IRPS$_L$, ARPS$_H$ and CYPS$_H$ groups were significantly elevated when compared with the controls (P < 0.05), and the largest mean number of IgA secreting cells in CYPS$_H$ and IRPS$_L$ groups was 128.9 and 120.9, respectively, and was 131.4 and 118.6 in APS$_L$, which in control group was 97.5 and 88.1. On day 60, the numbers of IgA secreting cells in IRPS$_L$ and CYPS$_H$ groups were significantly elevated when compared with controls (P < 0.05), and the largest mean number of IgA secreting cells in IRPS$_L$ group was 99.3, and 97.6 in APS$_L$, which in control group was 75.7 (Table 1).

Increased numbers of IgA secreting cells in cecum tonsils of treated chickens

On day 20 after the first vaccination, the numbers of IgA secreting cells in the cecum tonsil in the treatment groups were elevated when compared with the controls, and there was no significant difference (P > 0.05). On day 30, the numbers of IgA secreting cells in APS$_L$, IRPS$_H$, IRPS$_L$, ARPS$_H$ and CYPS$_H$ groups were significantly elevated when compared with the controls (P < 0.05), and the largest mean number of IgA secreting cells in IRPS$_L$ group was 138.3, and was 141.4 in APS$_L$, which in the control group was 108.7. On days 40 and 50, the numbers of IgA secreting cells in APS$_L$, IRPS$_L$, ARPS$_H$ and CYPS$_H$ groups were significantly elevated when compared with the controls (P < 0.05), and the largest mean number of IgA secreting cells in IRPS$_L$ group was 128.9 and 110.2, and was 122.5 and 108.8 in APS$_L$ group, which in the control group was 95.4 and 85.5. On day 60, the numbers of IgA secreting cells in APS$_L$, IRPS$_L$ and CYPS$_H$ groups were significantly elevated when compared with the controls (P < 0.05), and the largest mean number of IgA secreting cells in IRPS$_L$ group was 105.3, and for APS$_L$ group was 102.2, which in control group was 77.1 (Table 2).

Dynamic changes in serum ND virus-specific IgG antibody titers

On day 10 after the first vaccination, ND virus-specific IgG antibody titers among the 9 groups showed no significant difference (P > 0.05). For APS, on days 20, 30, 40, and 50, ND virus-specific IgG antibody titers in the APS$_L$ group were higher when compared with the controls significantly (P < 0.05). And on day 60, the titers in APS$_H$ and APS$_L$ groups were higher when compared with the controls significantly (P < 0.05); the antibody titer in APS$_L$ group was 8.9 $\log_2$, which in control group was 7.2 $\log_2$; the antibody titer increased   in   the APS$_L$ group was the

**Table 1.** Dynamic changes in the number of IgA secreting cells in the duodenum of vaccinated chickens.

Group	D_{10}	D_{20}	D_{30}	D_{40}	D_{50}	D_{60}
IRPS$_H$	72.3±11.2[a]	99.1±14.3[b]	119.6±16.1[b]	119.2±17.7[b]	97.8±13.6[b]	90.2±12.9[b]
IRPS$_L$	77.8±14.7[a]	105.5±15.2[b]	139.1±19.5[a]	127.1±18.2[a]	120.9±15.5[a]	99.3±12.6[a]
ARPS$_H$	75.9±10.9[a]	108.9±14.5[b]	135.5±18.3[a]	125.5±17.5[a]	114.7±14.5[a]	95.9±14.4[ab]
ARPS$_L$	73.6±11.6[a]	97.1±13.9[b]	118.8±15.4[b]	114.6±17.9[b]	95.7±13.2[b]	87.6±13.8[b]
CYPS$_H$	71.4±13.1[a]	110.6±15.9[ab]	136.4±19.8[a]	128.9±17.3[a]	115.2±14.9[a]	98.4±14.1[a]
CYPS$_L$	75.1±12.8[a]	98.7±13.5[b]	114.2±15.6[b]	110.8±16.8[b]	101.3±15.8[b]	84.7±13.2[b]
APS$_H$	79.2±14.2[a]	96.9±13.3[b]	133.2±21.9[a]	122.3±18.4[b]	105.1±15.1[b]	85.9±15.2[b]
APS$_L$	74.7±12.4[a]	117.3±17.4[a]	129.7±13.4[a]	131.4±18.9[a]	118.6±14.8[a]	97.6±14.6[ab]
Control group	74.9±13.7[a]	95.3±14.8[b]	101.4±17.7[b]	97.5±15.1[b]	88.1±14.3[b]	75.7±12.9[b]

Column data marked without the same superscripts differ significantly ($P<0.05$). D, day; APS, astragalus polysaccharide which is the positive control; IRPS, isatis root polysaccharide; ARPS, achyranthes root polysaccharide; CYPS, Chinese yam polysaccharide.

Table 2. Dynamic changes in the number of IgA secreting cells from cecal tonsils of vaccinated chickens.

Group	D_{10}	D_{20}	D_{30}	D_{40}	D_{50}	D_{60}
IRPS$_H$	69.7±12.9[a]	93.6±14.6[a]	128.7±17.9[a]	106.3±16.9[b]	99.1±15.4[b]	92.6±13.2[b]
IRPS$_L$	68.4±11.8[a]	102.8±15.2[a]	138.3±19.3[a]	128.9±16.2[a]	110.2±17.3[a]	105.3±16.1[a]
ARPS$_H$	70.3±12.7[a]	99.2±14.3[a]	127.8±14.8[a]	119.8±15.7[a]	107.9±16.6[a]	97.5±15.2[ab]
ARPS$_L$	60.8±11.1[a]	95.3±13.7[a]	117.9±15.6[b]	113.4±17.1[ab]	96.3±15.8[ab]	83.8±13.3[b]
CYPS$_H$	65.5±11.5[a]	98.4±14.1[a]	129.6±16.3[a]	120.7±16.4[a]	106.5±15.1[a]	99.9±12.4[a]
CYPS$_L$	67.9±12.2[a]	91.6±13.9[a]	112.3±16.8[b]	101.5±16.1[b]	92.4±14.2[b]	85.9±13.5[b]
APS$_H$	62.2±10.8[a]	100.7±14.9[a]	120.1±16.5[ab]	103.2±15.9[b]	97.6±14.5[b]	88.5±13.1[b]
APS$_L$	64.9±11.3[a]	94.5±13.8[a]	141.4±18.7[a]	122.5±15.5[a]	108.8±16.7[a]	102.2±14.3[a]
Control group	65.3±11.9[a]	89.9±13.6[a]	108.7±17.7[b]	95.4±15.3[b]	85.5±14.7[b]	77.1±12.8[b]

Column data marked without the same superscripts differ significantly ($P<0.05$). D, day; APS, astragalus polysaccharide which is the positive control; IRPS, isatis root polysaccharide; ARPS, achyranthes root polysaccharide; CYPS, Chinese yam polysaccharide.
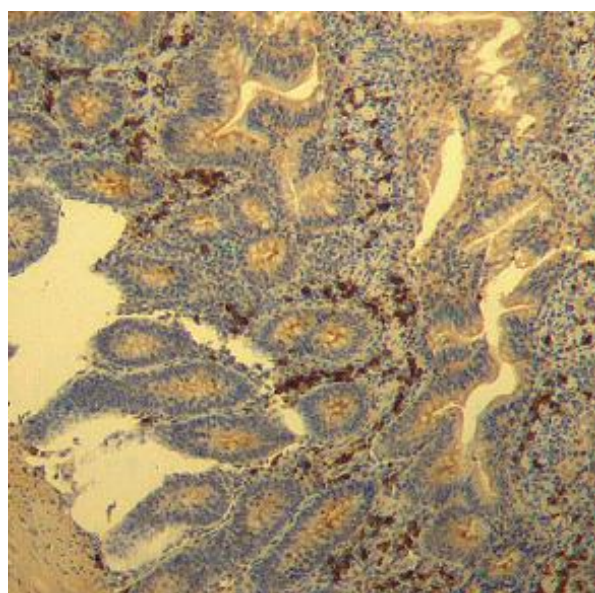
Figure 1. The section by Immunohistochemical staining which were observed using optical microscope. Distribution of SIgA positive cells which appears brown in the chicken duodenum and the section was observed at 400× magnification.
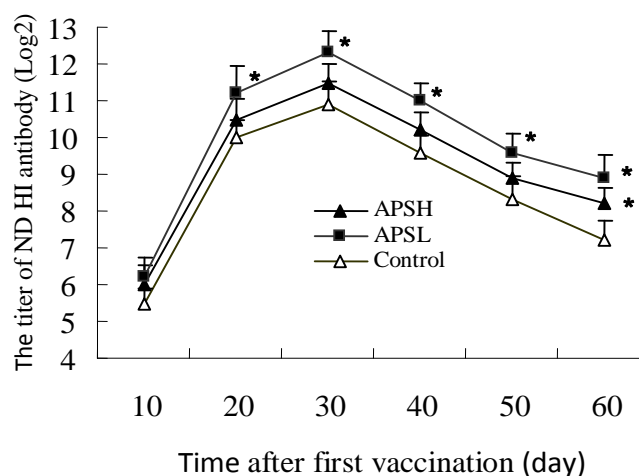
Figure 2. Dynamic changes of serum specific IgG antibody titers (Log_2) in APS (positive control) and control groups. Data represent the mean ± SD. *$P<0.05$ compared with controls. ND, Newcastle disease; APS, astragalus polysaccharide; APS$_H$, high dose astragalus polysaccharide; APS$_L$, low dose astragalus polysaccharide.

maximum (1.7 log_2) when compared with the controls (Figure 2). For IRPS, on days 20, 40, and 50, the titers in

$IRPS_L$ group were higher when compared with the controls significantly (P < 0.05). On days 30 and 60, the titers in $IRPS_H$ and $IRPS_L$ groups were higher when compared with controls significantly (P < 0.05). And on days 60, the antibody titer in $IRPS_L$ group was 8.8 log_2, which in control group was 7.2 log_2; the antibody titer increased in $IRPS_L$ group was the maximum (1.6 log_2) when compared with the controls (Figure 3). For ARPS, on days 20, 30, 40, 50, and 60, the titers in $ARPS_H$ group were higher when compared with the controls (P < 0.05). And on days 60, the antibody titer in $ARPS_H$ group was 8.5 log_2, which in control group was 7.2 log_2; the antibody titer increased in the $ARPS_H$ group was the maximum (1.3 log_2) when compared with the controls (Figure 4). For CYPS, on days 20, 30, 40, 50, and 60, the titers in the $CYPS_H$ group were higher when compared with controls significantly (P < 0.05). And on days 60, the antibody titer in $CYPS_H$ group was 8.4 log_2, which in control group was 7.2 log_2; the antibody titer increased in CYPSH group was the maximum (1.2 log_2) when compared with controls (Figure 5). It showed that IRPS is the most similar to APS in raising ND virus-specific IgG antibody titers.

DISCUSSION

The mucosal immune system is equipped with unique innate and acquired defense mechanisms, which provide a first line of protection against ingested infectious agents (Mick and Karin, 2006). Secretory IgA is the major antibody isotype present in mucosal secretions and has many functions, both direct and indirect, that prevent infective agents such as bacteria and viruses from breaching the mucosal barrier (Egmond et al., 2001; Russell and Sibley, 1999). Therefore, IgA secreting cells are important for the protection of mucosal surfaces. Changes in the numbers of IgA secreting cells are one of the standards used to estimate mucosal immunity. In this study, the presence of positive IgA secreting cells was detected from duodenum and cecum tonsils of chickens by immunohistochemistry. We found that the numbers of positive IgA secreting cells per unit area from tissues of treatment groups were much higher than those from control groups at most time points, especially in the APS_L, $IRPS_L$, $ARPS_H$ and $CYPS_H$ treatment groups. This suggests that APS, IRPS, ARPS and CYPS might promote the differentiation   and proliferation of IgA secreting cells in the intestinal mucosa of chickens. This demonstrated that Chinese herbal polysaccharides could effectively stimulate mucosal immune responses to resist external microbial invasion. Interestingly, we found that the effects of APS and IRPS at low doses were better than ARPS and CYPS at high doses. This phenomenon concurs in part with a study by Zhang et al. (2007), where two compound adjuvants (cMIA I and cMIA II) promoted IgA secreting cells and intestinal intraepithelial lymphocytes in chickens vaccinated with attenuated Newcastle-disease vaccine.

Figure 3. Dynamic changes of serum specific IgG antibody titers (Log_2) in IRPS and control groups. Data represent the mean ± SD. *P<0.05 compared with controls. ND, Newcastle disease; IRPS, isatis root polysaccharide; $IRPS_H$, high dose isatis root polysaccharide; $IRPS_L$, low dose isatis root polysaccharide.
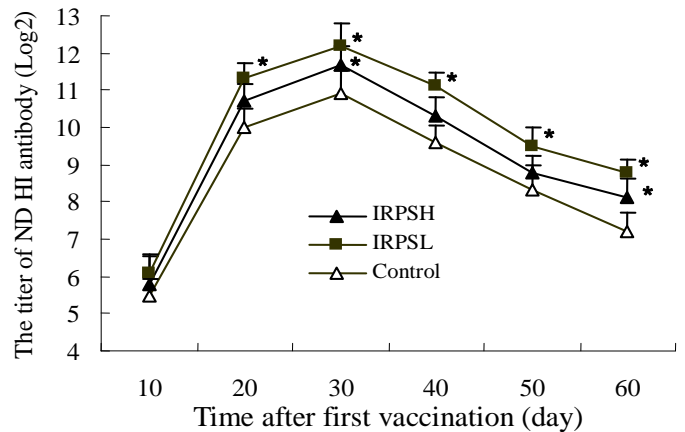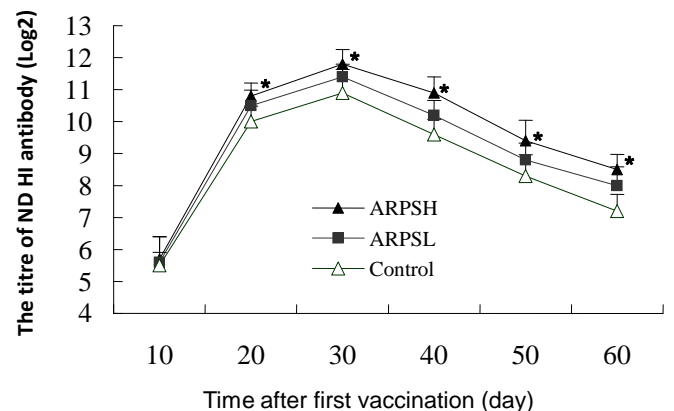

Figure 4. Dynamic changes of serum specific IgG antibody titers (Log_2) in ARPS and control groups. Data represent the mean ± SD. *P<0.05 compared with controls. ND, Newcastle disease; ARPS, achyranthes root polysaccharide; $ARPS_H$, high dose achyranthes root polysaccharide; $ARPS_L$, low dose achyranthes root polysaccharide.

Merz et al. (1981) reported that humoral immune responses played important roles in the host's defense against ND virus infection. The specific antibodies could neutralize or inactivate the free virus by binding to virus surface glycoproteins, thus inhibiting the attachment of virus to cells, and blocking viral spread. The dynamic changes of specific serum IgG antibody titers reflect the state of humoral immunity in the animals. Our results showed that the antibody titers in most treatment groups at many time points were significantly higher than in the control group. Titers in the APS_L, $IRPS_L$, $ARPS_H$ and $CYPS_H$ groups at five time points were significantly higher than in controls, indicating that low dose IRPS and   high
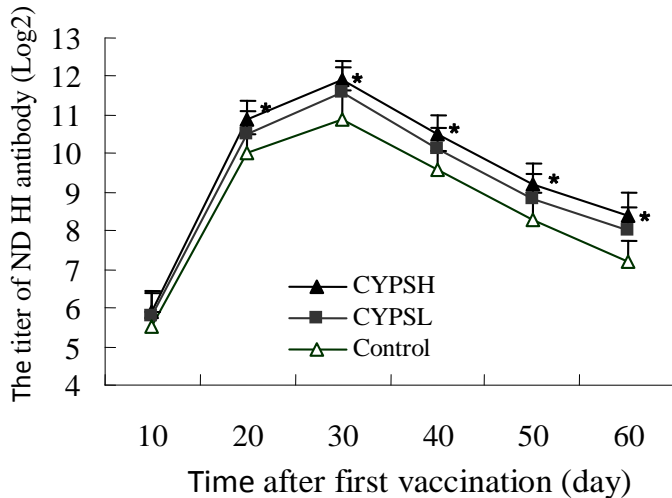
Figure 5. The dynamic changes of serum specific IgG antibody titers (Log_2) in CYPS and control groups. Data represent the mean ± SD. *$P<0.05$ compared with controls. ND, Newcastle disease; CYPS, Chinese yam polysaccharide; $CYPS_H$, high dose Chinese yam polysaccharide; $CYPS_L$, low dose Chinese yam polysaccharide.

dose ARPS and similar to APS,CYPS had the best effect on enhancing humoral immunity. Antibody titers in low dose APS and IRPS and high dose ARPS and CYPS chickens up to 60 days old were still higher than 8.4 Log2, while the titer in control group was 7.2 Log2. This indicated that APS, IRPS, ARPS and CYPS at suitable doses could maintain higher antibody titers, because of a slower decline of antibody titer. Gu et al. (2005) reported that Chinese herbal medicine compound polysaccharides could promote the development of immune organs in chickens. The main immune organ for specific antibody production is the thymus and bursa of Fabricius (Alam et al. 1997). Thus, these three Chinese herbal crude polysaccharides could strengthen humoral immunity in vaccinated chickens through promoting development of immune organs.

These results showed that immune-enhancing effects of the three different crude polysaccharides are very similar to APS. In our previous preliminary test, a variety of traditional Chinese medicine were selected and extracted to observe the immunomodulatory effects on mice, and then these crude polysaccharides which had the better effect were selected to observe its immune-enhancing effect in chickens, when compared with APS. This may be the reasons why the experimental results are very similar, but it indicated that the suitable doses of these crude polysaccharides were not the same, and the effect of IRPS which was most similar to APS at low doses were slightly better than ARPS and CYPS at high doses. Similar research also verified the remarkable potential benefits of crude polysaccharides derived from Chinese medicines (Chen et al., 1997; Sun et al., 2005).

Conclusion

This study confirmed that low dose IRPS and high dose ARPS and CYPS could significantly promote the differentiation and proliferation of IgA secreting cells in the intestinal mucosa and increase serum ND virus-specific IgG antibody titers, and thus, enhance mucosal and humoral immune responses. It takes longer time to inhibit the multiplication of virus when compared with antiviral drug, but Chinese herbal crude polysaccharides has the advantages of natural, safe, less toxic or side effect at suitable dose, because the antiviral efficacy of these Chinese herbal crude polysaccharides is achieved through enhancing the body's immune system. Therefore, Chinese herb polysaccharides should be used for the prevention of viral diseases, rather than treatment. These three crude polysaccharides may form the basis for a new immune potentiating drug in the domestic animal and poultry industry. The dosage used is an important factor and must be considered in the development of a Chinese herbal medicinal immune potentiating drug. Further study on the mechanism of protective vaccination effects of the three Chinese herbal polysaccharides is underway.

REFERENCES

Alam KMT, Lslam MA, Rahman MM (1997). Antibody response and challenged against Newcastle disease. Bangladesh Vet. J. 31:23-27.
Chen YQ, Chen JX, Zhang TS (1997). The antitumor effect and the influence on immunity function of the four polysaccharides. Chin. J. Cancer. 3:198-200.
Cho WC, Leung KN (2007). *In vitro* and *in vivo* immunomodulating and immunorestorative effects of *Astragalus membranaceus*. J. Ethnopharmacol. 113(1):132-141.
Cui W, Wu GX, Zhang ZL (2011). Effects of *Achyranthis bidentatae Radix* and *Achyranthes bidentata* Polysaccharides on Enhancing Immune Function. Chin. J. Exp. Trad. Med. Formul. 16:141-143.
Egmond MV, Damen CA, Spriel ABV (2001). IgA and IgA Fc receptor. Trends Immunol. 22:205-211.
Gu XL, Li HQ, Wang JD (2005). Effects of Compound Polysaccharide Extracted from Traditional Chinese Medical Herbs on the Immunity Function in Chickens. Scientia Agric. Sinica 4:813-820.
Hu YL (1997). Progress in the study of immunopharmacology of Chinese herbal medicine. Chin. J. Immunol. 3:96-98.
Liu XP, MA Z, Wang XY,Wang Y (1994). Studies on Huangqi polysaccharide oral liquid. J. Chin. Med. Mater. 6:40-43.
Lu XT, Dai JH, Liao M, Liu N (2003). Advances of studies on immunoregulative activities of polysaccharides. Prog. Vet. Med. 24:10-12.
Ma HD, Deng YR, Tian Z, Lian ZX (2012). Traditional Chinese Medicine

and Immune Regulation. Clin. Rev. Allergy Immunol. 24:1-4.

Merz DC, Scheid A, Choppin P (1981). Immunological studies of the functions of paramyxovirus glycoprotein. Virology 28:208-221.

Mick B, Karin H (2006). The postnatal development of the mucosal immune system and mucosal tolerance in domestic animals. EDP Sci. 37:443-453.

Russell MW, Sibley DA (1999). IgA as an anti-inflammatory regulator of immunity. Oral Dis. 5:55-57.

Sun HL, Miao DY, Gong YM, Zhang PJ (2005). Effect of extractive amylose from tragacanth, rock alga and cole on the immunity with ND live vaccine in chickens. Chin. J. Prev. Vet. Med. 5:205-208.

Sun, Xie B (2011). Progress in pharmacological study of Chinese yam. Trad. Chin. Drug Res. Clini. Pharmacol. 3:353-355.

Thekisoe MMO, Mbati PA, Bisschop SPR (2004). Different approaches to the vaccination of free ranging village chickens against Newcastle disease in Qwa-Qwa. South Afr. Vet. Microbiol.101:23-30.

Veterinary Pharmacopoeia Commission of the People's Republic of China (2000). Veterinary pharmacopoeia of the People's Republic of China. Chemical Industrial Press. Beijing, China. pp. 72-73.

Xue YL (1985). Handbook of laboratory experiments in plant physiology. Shanghai Science and Technology Press, Shanghai, China, pp. 136-138.

Xie XQ (2000). Exploitation and application of new-type preparation of Chinese herbal medicine. People Sanitary Press, Beijing, China. pp. 377-383.

Yang Q, Lian GJ, Huang GQ (2002). Effect of cysteamine on the modulation of sIgA cells and intraepithelium lymphocytes in chicken intestine. J. Nanjing Agric. Univ. 25:89-92.

Yon ZT, Lina ZH, Peter C (2006). Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. Carbohydr. Res. 341:2261-2269.

Zhang XF, Zhang XW, Yang Q (2007). Effect of compound mucosal immune adjuvant on mucosal and systemic immune responses in chicken orally vaccinated with attenuated Newcastle disease vaccine. Vaccine 25:3254-3262.

Zhao YL, Wang JB, Shan LM, Jin C, Ma L, Xiao XH (2008). Effect of Radix isatidis polysaccharides on immunological function and expression of immune related cytokines in mice. Chin. J. Integr. Med. 14(3):207-211.

Full Length Research Paper

Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district

Alemu Aylate Ayano[1]*, Fikiru Hiriko[2], Alemante Molla Simyalew[1] and Aster Yohannes[3]

[1]School of Veterinary Medicine, Wolaita Sodo University, Wolaita Sodo, Ethiopia.
[2]School of Veterinary Medicine, Wollo University, Dessie, Ethiopia.
[3]Holeta Agricultural Research Center, Holeta, Ethiopia.

A cross-sectional study was carried out to determine the prevalence of subclinical mastitis in lactating dairy cows from August 10, 2011 to May 25, 2012 in three purposively selected commercial dairy farms in Holeta district, Ethiopia. The study was carried out through field screening surveys by California mastitis test for each quarter milk sample, followed by bacteriological examination to identify the causative agents of intra-mammary infection. A total of 546 milking cows were examined, out of which 224 (41.02%) were found positive for subclinical mastitis on the basis of California mastitis test. Milk samples collected from 224 positive cows were subjected to microbiological culture for the isolation of pathogenic bacteria. One hundred eighty three (81.7%) of the samples were found positive for bacterial isolation. The major isolate pathogens were *Staphylococcus aureus* (13.8%), *Streptococcus uberis* (12.1%), *Staphylococcus epidermidis* (11.7%), *Escherichia coli* (11.6%), *Streptococcus dysagalactiae* (10.6%), *Pseudomonas aeruginosa* (9.7%), *E. coli* $O_{157:H7}$ (6.9%), *Micrococcus* species (6.5%) and *Streptococcus agalactiae* (6.4%) and others (10.7%). Subclinical mastitis is endemic in Holeta dairy farms and thereby necessary measures are needed to be taken to prevent further losses.

Key words: California mastitis test, bacteriological culture, prevalence, subclinical mastitis.

INTRODUCTION

Despite many years of research, mastitis subclinical remains the most economically damaging and zoonotic potential disease for dairy industry and consumers worldwide irrespective of the species of animal (Ojo et al., 2009). Economic losses caused by mastitis include value of discarded milk, reduction in quality of milk and cost of treatment (Radostits et al., 2007). Bacterial contamination of milk from affected cows may render it unsuitable for human consumption by causing food poisoning or inter-ference with manufacturing process or in rare cases, provides mechanism of spread of disease to humans. Zoonotic diseases potentially transmitted by raw cow milk include brucellosis, caseous lymphadenitis, leptospirosis,

listeriosis, melioidosis, Q-fever, staphylococcal food poisoning, toxoplasmosis and tuberculosis (Mungube et al., 2005; Radostits et al., 2007).

The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, sub-clinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk et al., 2003). Previous studies conducted in different countries indicated the distribution and economic importance of the disease. Contreras et al. (1997) from Spain, Moshi et al. (1998) from Tanzania, Ameh and Tari (2000) from Nigeria, Ndegwa et al. (2000) from Kenya and Kozacinski et al. (2002) from Croatia reported different prevalence rates of mastitis in dairy cattle. The disease has been reported by several authors in different parts of Ethiopian country (Mungube et al., 2005; Lakew et al., 2009; Gebreyohannes et al., 2010; Megersa et al., 2010). Several of these studies have shown the occurrence of a range of

*Corresponding author. E-mail: ayanoalemu@yahoo.com. Tel: +251-913838769.

mastitis causing bacteria, indicating *Staphylococcus*, *Escherichia coli* and *Streptococcus* as dominant and pathogenic species. Some authors (Mungube et al., 2005) reported a substantial economic loss in Ethiopian highland crossbred dairy cows due to subclinical mastitis.

Subclinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT), the modified white side test, somatic cell count, pH, and catalase tests. These tests are preferred as the screening tests for subclinical mastitis as they can be used easily, yielding rapid, as well as satisfied results (Joshi and Gokhale, 2006).

In some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Such information is important to envisage when designing appropriate strate-gies that would help to reduce its prevalence and effects (Mekebib et al., 2009; Megersa et al., 2010). This study aimed: (i) to evaluate the prevalence of subclinical mastitis in apparently healthy dairy cows in Holeta district, (ii) to determine the most frequency of intra-mammary infection, causative agents, and (iii) to evaluate associated risk factors affecting on subclinical mastitis.

MATERIALS AND METHODS

Study area

The study was conducted in dairy farms of Holeta town located 45 km away from Addis Ababa in the south west direction, 9° 3' N and 38° 30' E, at an altitude of 2,400 m above sea level in central highlands. The area is characterized by mild subtropical weather with minimum and maximum temperature ranging from 2 to 9°C and 20 to 27°C, respectively. The area receives annual rainfall of 1060 mm (CSA, 2010).

Study population

A total of 546 dairy cows were examined in three different dairy farms in Holeta town. The dairy cows were distributed according to breed (136 Holstein Friesian breed, 150 Jersey and 260 Holstein × Borena cross breed cows), age (322 cows aged less than 6 years young and 224 cow aged greater than or equal to 6 years old). All dairy cows had no clinical symptoms. They lived nearly under the same conditions of breeding from the habitat, hygiene and feeding systems. All animals were subjected to clinical and physical exami-nations, with special interest towards the udder and teats. At the time of each examination, the breed of the cow, age of the cow, health status of the mammary glands and the respective farm names were recorded.

Study design, sample size and sampling method

A cross sectional study was conducted. Three dairy farms were purposively selected for their ease of accessibility. Simple random sampling technique was followed to select the study animal, and the desired sample size was calculated according to the formula given by Thrusfield (2007). Milk samples were taken from apparently healthy animals in these dairy farms. A total of 546 dairy cows were examined in three different dairy farms in Holeta district, Ethiopia,

and spread out over ten months (during the period from August 10, 2011 to May 25, 20112).

Physical examination of mastitis

Udder attachment, parity number, any physical abnormalities such as swelling of the udder, presence of lesions, anatomical malformations and tick infestation were recorded. The milk was examined for its color, odor, consistency and other abnormalities prior to milking.

California mastitis test (CMT)

The California mastitis test was carried out as described by Hogan et al. (1999) and Quinn et al. (2004). A squirt of milk, about 2 ml from each half was placed in each of 2 shallow cups in the CMT paddle. An equal amount of the commercial CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 s. Based on the thickness of the gel formed by CMT reagent-milk mixture, test results were scored as 0 (negative/trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive). Positive CMT-cows were defined as having at least one CMT-positive quarter.

Milk sample collection, handling and transportation

Aseptic procedures for collecting quarter milk samples as described by Hogan et al. (1999), Sears et al. (1991) and Quinn et al. (2004) were followed. The time chosen for milk sample collection was before milking. Udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with cotton alcohol pads. A separate pledged of cotton was used for each teat. The first streams of milk were discarded and 10 ml of milk was collected into horizontally held vial. After collection, the sample was placed in an icebox and transported to the laboratory for analysis.

Microbiological culture

Each positive CMT milk sample was collected under aseptic conditions in a sterile screw caped bottle numbered to identify the particular quarter and cow. All milk samples were sent directly to the laboratory, with a minimum delay for routine culture techniques. Milk samples were cultured onto 10% sheep blood agar and MacConkey agar plates according to Athar (2006), Coulon et al. (2002) and Quinn et al. (2004). Suspected colonies were identified morphologically, microscopically and biochemically according to National Mastitis Council (NMC) (2004), Iqbal et al. (2004) and Quinn et al. (2004). Cultures with fine bacterial growth were considered as positive and cultures with no visible growth taken as negative, but polluted cultures with disturbed media were considered as contaminated according to Shakoor (2005). Pure isolates of *E. coli* were inoculated into 10 ml of brain-heart infusion (BHI) broth (Oxoid Ltd, Basingstoke, Hampshire, UK), supplemented with yeast extract (Oxoid) followed by incubation at 37°C for 8 h, to further identify serotype of *E. coli* according to Quinn et al. (2004).

Statistical analysis

The data was compiled and analyzed with Statistical Package for Social Sciences (SPSS statistical package version 17). Prevalence estimation of commonly isolated pathogens in Holeta town dairy farms was determined using standard formulae (that is, the number

of positive animals/samples divided by the total number of animals/samples examined). Descriptive statistics such as percent-tages and frequency distributions was used to describe/present the nature and the characteristics of the data.

RESULTS

California mastitis test (CMT)

Out of 546 lactating cow examined, 224 (41.02%) were diagnosed with subclinical mastitis in the study area, out of which 130 (58%), 58 (26%) and 36 (16.1%) were from A, B and C dairy farms, respectively. Significant difference in mastitis prevalence (P < 0.05) was observed among studied farms (Table 1). The prevalence of subclinical mastitis did not vary among age group. However, relatively higher prevalence of subclinical mastitis was recorded in adult (46.42%) followed by young age group (37.3%). There was no significant difference (P > 0.05) in infection among age groups (Table 1). Prevalence of subclinical mastitis did not vary along with the lactation stages of animal, but relatively highest prevalence was seen in animals at mid lactation stage (50%), followed by animals at late lactation (47.2%) and a least in early lactation stage (37.5%). The result of statistical analysis revealed no significant difference (P > 0.05) among the lactation stages (Table 1).

Mastitis causing pathogens

Out of 224 positive samples for subclinical mastitis, only 183 (81.7%) samples showed growth on 10% sheep blood agar and 28 (12.5%) samples showed no growth, and about 13 (5.8%) were contaminated samples. From 183 culture positive samples, a total of 596 bacteria of seven genera were isolated. The relative prevalence of various bacterial species isolated from subclinical mastitis cases are shown in (Table 2). The most prevalent isolated pathogens were *Staphylococcus aureus* (13.8%), *Streptococcus uberis* (12.1%), *Streptococcus epidermidis* (11.7%), *E. coli* (11.6%), *Enterobacter aerogenes* and *Klebsiella pneumonia* (10.7%), *Streptococcus dysagalactiae* (10.6%) and *Pseudomonas aeruginosa* (9.7%). Other bacterial isolates includes *E. coli* O157:H7 (6.9%), *Micrococcus* species (6.5%) and *S. agalactiae* (6.4%).

DISCUSSION

The present epidemiological study was applied through combination of the CMT with bacteriological cultures. Thus, subclinical mastitis was defined as a state when mammary glands without clinical abnormalities give apparently normal milk but was bacteriologically positive and with positive CMT (Mungube et al., 2005).

Karimuribo et al. (2006) concluded the CMT is still the superior screening diagnostic aid for subclinical mastitis, while bacteriological examination is still the most suitable technique of diagnosis. This study detected the subclinical mastitis in 224 out of 546 milking cows examined, which result in a prevalence of 41.02% subclinical mastitis in dairy farms of Holeta District. This result is in agreement with previous studies by Mekebib et al. (2009), Sori et al. (2005), Workineh et al. (2002) and Girma (2010) who reported prevalence of 34.8, 40.6, 38.6 and 34.4%, respectively. However, the prevalence of subclinical mastitis in this study is relatively higher than previous 23.0% by Biffa et al. (2005) and 9.81% by Lakew et al. (2009) in Southern Ethiopia and Khartoum, respectively. Because mastitis is a complex disease involving interactions of several factors, mainly of management, environment, and factors relating to animal and causative organisms, its prevalence is expected to vary from place to place.

All 224 CMT positive subclinical samples were cultured on bovine blood agar and accordingly, 183 (81.7%) were found culture positive. The failure to isolate the bacteria from the CMT positive milk samples could be partly asso-ciated with spontaneous elimination of infection, low con-centration of pathogens in the milk, intermittent shedding of pathogen, and intracellular location of pathogens and presence of inhibitory substance in the milk (Radostits, 2007). A total of 596 isolates of seven (7) different microbial species were isolated.

The present study also revealed a close positive relationship between isolation of bacteria from mastitic milk samples and California mastitis test. As almost all milk samples were positive to CMT, specific bacteria were isolated. This means that CMT was a good diagnostic tool in the detection of sub-clinical mastitis; hence it could be most the reliable test to be conducted to investigate sub-clinical mastitis in the dairy farms. On the other hand, the culture method may be used to confirm and aid proper treatment (Tefera, 2001; Barnouin et al., 2005; Bitew et al., 2010; Bekele and Molla, 2001).

Mastitis has a multifactorial nature that predominates with a clear interaction between host, agent and environ-ment (Thusfield, 2007). For this reason, the studied factors here were determined as breed, age and lactation stage (Riekkerink et al., 2008). Considering the breed factor, it was found that the Holstein-Borena breed (50%), all kept in farm A, were found more susceptible than Jersey breed (38.7%), all kept B, and Holstein-Frisian breed cows all kept in C (26.5%) were found least susceptible. Thus, breed difference was found to be statistically significant (P < 0.05). The high prevalence of subclinical mastitis in farm A could be associated with breed susceptibility, poor hygienic and managemental conditions. It was observed that subclinical mastitis frequently encountered in the examined dairy cows were more common in middle (50%) and late lactation stage (47.62%) than early lactation stage (36.7%). Hence, regime could be possibly among the major factors contributing to high prevalence at middle stage. During a dry period, due to low bactericidal

Table 1. Association between some of factors with occurrence of subclinical mastitis.

Risk factor		Cow		Prevalence (%)	X^2	P value
		Total	Infected			
Age	Young[a]	322	120	37.3	2.299	0.21
	Adult[b]	224	104	46.42		
Farms/breed	A[c]	260	130	50	10.454	0.03
	B[d]	150	58	38.7		
	C[e]	136	36	26.5		
Lactation stage	Early[f]	360	132	36.7	0.133	0.13
	Mid[g]	144	72	50		
	Late[h]	42	20	47.62		

[a]Young: < 6 years, [b]Old: ≥ 6 years, [c]A: Holeta agricultural research center dairy farm (Holstein × Borena breed), [d]B: Ada'a Berga agricultural research center dairy farm (Jersey breed), [e]C: Holeta cattle genetic improvement center dairy farm (Holstein-Friesian breed), [f]Early: 1 to 120 days of lactation, [g]Mid: 120 to 240 days of lactation, [h]Late: >240 days of lactation.

Table 2. Frequency of mastitis causing pathogen isolated from subclinical mastitis in dairy cows.

Species of bacteria identified	No. of isolates/farms			Total No. of isolate	Percentage
	A[c]	B[d]	C[e]		
E. coli	45	15	9	69	11.6
E. coli O157:H7	31	10	0	41	6.9
S. aureus	28	33	21	82	13.8
M. species	19	12	8	39	6.5
S. epidermidis	46	8	16	70	11.7
S. uberis	40	14	18	72	12.1
S. dysagalactiae	33	19	11	63	10.6
S. agalactiae	22	8	8	38	6.4
P. aeruginosa	23	24	11	58	9.7
Others*	25	27	12	64	10.7
Total	312	170	114	596	100

*Other include *E. aerogenes* and *K. pneumonia*, [c]A: Holeta agricultural research center dairy farm (Holstein × Borena breed), [d]B: Ada'a Berga agricultural research center dairy farm (Jersey breed), [e]C: Holeta cattle genetic improvement center dairy farm (Holstein-Friesian breed).

and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply. The increased prevalence of mastitis with advancing lactation number agrees with the findings of previous investigators (Harmon, 1994; Radostits et al., 2007; Zerihun, 1996).

The prevalence of mastitis with age seen in this study is similar to reports by Biffa et al. (2005). The high prevalence of subclinical mastitis in aged multiparous animals might be due to increase in teat patency and frequency of previous exposure (Harmon, 1994).

In present study, most major pathogen isolated were *S. aureus* (13.8%), which was not similar with reports by Sori et al. (2005), Sharif et al. (2009) and Mekebib et al.

(2009). This variation may be due to season, managemental conditions at the farm, area, difference in sample handling in the laboratory and use of antibiotics. *E. coli* identified in the present study (11.6%) was not similar with reports by Mekebib et al. (2009), Bitew et al. (2010) and Sori et al. (2005) with an isolation rate of 43.13, 20.3 and 26.57%, respectively. This lower report of isolates might be partly associated with effective udder washing and drying, post milking teat dip and keeping cleanness of washing towels. The present study also identified a low prevalence of *Micrococcus* spp. (8.15%) and *Corynebacterium bovis* (1.7%), which was in-line with findings of Workineh et al. (2002), Bitew et al. (2010) and

Sori et al. (2005). *S. agalactiae* was isolated with a proportion of 6.4%. The result of present study was similar with those described by Lakew et al. (2009) and Bitew et al. (2010) who reported 4 and 8.8%, respectively.

The prevalence of streptococcal isolation during this study (29.03%) was lower than that reported for the same species by Okeke et al. (2005) (80.95%) in dairy cows. The lower isolation rate in this study might be associated with the wide spread use of penicillin in the area for treatment of mastitis. It has been recognized that mastitis caused by *Streptococcus* species is susceptible to eradication via use of penicillin. *S. uberis* isolation (12.1%) in this study was higher than that reported by Mekebib et al. (2009) (6.53%), but lower than that of Zerihun (1996) and Iqbal et al. (2004) who reported 27 and 49.98%, respectively.

In this study, the prevalence of subclinical mastitis was accompanied with analysis of different risk factors including farm and breed differences, lactation stages and isolation of major bacterial pathogens in subclinical mastitis cows. Cross-breed was more stuck by subclinical mastitis than Jersey and Holstein-Frisian breeds. Aged cows showed most sensitivity for subclinical mastitis. Mid lactation stage was seen with higher prevalence.

CONCLUSION AND RECOMMENDATIONS

In a spite of a large research efforts aimed to gain prevalence and to develop a new control tools for mastitis, the subclinical occurrence of the mastitis remains a substantial problem for dairy producers. The result of the present study indicated a relatively high prevalence of subclinical mastitis in dairy cattle of the study area. The relatively high prevalence reported in this study clearly indicated lack of strategic control measures against the disease, as well as poor surveillance measures. Lack of maintenance of strict hygiene and good sanitary environment may be contributory factors in the cause of subclinical mastitis. It is therefore important that farmers should ensure strict personal hygiene and that of animals, and general sanitary condition of the farms should be improved and maintained. Furthermore, all dairy producers should know that early detection of intra-mammary infection is important for selecting and implementing proper therapy. Unfortunately, most infections are not detected until they become clinical, and by then, extensive and costly damages could result. Routine milk cultures should be an ongoing part of any mastitis control program. The sampling strategies for any ongoing program require the input of the herd veterinarian, as well as herd management.

ACKNOWLEDGEMENTS

REFERENCES

Ameh JA, Tari LS (2000). Observation on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri. Small Rumin. Res. 35:1-5.

Barnouin J, Bord S, Bazin S, Chassagne M (2005). Dairy management practices associated with incidence rate of clinical mastitis in low somatic cell score herds in France. J. Dairy Sci. 88:3700-3709.

Bekele T, Molla B (2001). Mastitis in lactating camels (Camels dromedarus) in Afar region, Northeast Ethiopia. Berl. Munch. Tierarztl. Wochenschr. 114:169-172.

Biffa D, Debela E, Beyene F (2005). Prevalence and risk factors of mastitis in lactating dairy cows in Southern Ethiopia. Int. J. Appl. Res. Vet. Med. 3(3):189-198.

Bitew M, Tafere A, Tolosa T (2010). Study on bovine mastitis in dairy farms of Bahir Dar town and its environ. J. Anim. Vet. Adv. 9:2912-2917.

Contreras A, Corrales JC, Sanchez A, Sierra D (1997). Persistence of Caprine intra-mammary pathogens throughout lactation. J. Dairy Sci. 80:2815-2819.

Coulon JB, Gasqui P, Barnoun J, Ollier A, Pardel P, Dominique P (2002). Effect of mastitis and related germ yield and composition during naturally occurring udder infections in dairy cows. Anim. Res. 51:383-393.

CSA (2010). Ethiopian Agricultural Sample Enumeration (EASE), Addis Ababa, Ethiopia.

Gebreyohannes YT, Regassa FG, Kelay B (2010). Milk yield and associated economic losses in quarters with subclinical mastitis due to *Staphylococcus aureus* in Ethiopian crossbred dairy cows. Trop. Anim. Health Prod. 42:925-931.

Girma DD (2010). Study on prevalence of bovine mastitis on cross breed dairy cow around Holeta areas, West Shewa Zone of Oromia, Ethiopia. Glob. Vet. 5(6):318-323.

Harmon RJ (1994). Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci. 77(7):2103-2112.

Iqbal M, Khan MA, Daraz B, Saddique U (2004). Bacteriology of mastitic milk and *in vitro* antibiogram of the isolates. Pak. Vet. J. 24:161-164.

Joshi S, Gokhale S (2006). Status of mastitis as an emerging disease in improved and periurban dairy farms in India. Ann. N. Y. Acad. Sci. 1081:74-83.

Karimuribo ED, Fitzpatrick JL, Bell CE, Swai ES, Kambarage DM, Ogden NH, Bryant MJ, French NP (2006). Clinical and subclinical mastitis in smallholder dairy farms in Tanzania. Risk intervention and knowledge transfer. Prev. Vet. Med. 74:84-98.

Kozacinski LM, Iladziosmanovi T, Majic IK, Jole, Cvrtila Z (2002). Relationships between the results of mastitis tests, somatic cell counts and the detection of mastitis agents in milk. Praxis Vet. 57:255-260.

Lakew M, Tolosa T, Tigre W (2009). Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. Trop. Anim. Health Prod. 41:1525-1530.

Megersa B, Chala T, Abunna F, Regassa A, Berhanu M, Etana D (2010). Occurrence of mastitis and associated risk factors in lactating goats under pastoral management in Borana, Southern Ethiopia. Trop. Anim. Health Prod. 42:1249-1255.

Mekebib B, Furgasa M, Abunna F, Megersa B, Furgasa A (2009). Bovine mastitis prevalence, risk factors and major pathogens in dairy farms of Holeta Town, Central Ethiopia. Vet. World 13(9):397-403.

Moshi NG, Kitaro GC, Minga UM (1998). Prevalence of mastitis in dairy goats in some selected farms in Morgoro and Arusha, Tanzania. Tanzan. J. Agric. Sci. 1:173-180.

Hogan SJ, Gonzalez RN, Harmon JR, Nickerson SC, Oliver SP, Pankey JW, Smith LK (1999). Laboratory Handbook on Bovine Mastitis. In: Hoard WD (ed.), National Mastitis Council, Inc., Fort Atkinson, USA.

Mungube ED, Tenghagen BA, Regassa F, Kyule MN, Shiferaw Y, Kassa T, Baumann MPO (2005). Reduced milk production in udder quarters with subclinical mastitis and associated economic losses in crossbred dairy cows in Ethiopia. Trop. Anim. Health. Prod. 37(5):

503-512.

National Mastitis Council (2004). Microbiological procedures for the diagnosis of udder infection. 3rd ed., National Mastitis Council Inc. Arlington, VA.

Ndegwa EN, Mulei CM, Munyna SJ (2000). The prevalence of subclinical mastitis in dairy goats in Kenya. J. S. Afr. Vet. Assoc. 71(1):25-27.

Ojo OE, Oyekunle MA, Ogunleye AO, Otesile EB (2009). *Escherichia coli*, O157:H7 in food animals in part of South-Western Nigeria. Prevalence and *in vitro* antimicrobial susceptibility. Trop. Vet. 26(3):23-30.

Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect. Dis. 5(8):481-493.

Quinn PJ, Carter ME, Markey B, Carter GR (2004). Clinical Veterinary Microbiology. London Wild life Publisher. pp. 95-101.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007). Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th ed., Saunders Elsevier, Spain. pp. 1045-1046.

Riekkerink RGMO, Barkema HW, Kelton DF, Scholl T (2008). Incidence rate of clinical mastitis on Canadian dairy farms. J. Dairy Sci. 91:1366-1377.

Sears PW, Wilson DJ, Gonzalez RN, Hancock DD (1991). Microbiological results from milk samples obtained pre-milking and post-milking. J. Dairy Sci. 74:4183-4188.

Shakoor A (2005). Preparation and evaluation of *Staphylococcus aureus* vaccines for the control of mastitis in dairy buffaloes (*Bubalus bubalis*). PhD Dissertation, Department of Veterinary Clinical Medicine and Surgery, College of Agriculture, Faisalabad, Pakistan. pp. 34-40.

Sharif A, Umer M, Muhammad G (2009). Mastitis control in dairy production. J. Agri. Soc. Sci. 5:102-105.

Sori H, Zerihun A, Abdicho S (2005). Dairy cattle mastitis in and around Sebeta, Ethiopia. Int. J. Appl. Res. Vet. Med. 3:332-338.

Tefera G (2001). Prevalence of mastitis at Alemaya University dairy farm. J. Ethiop. Vet. Assoc. 3:17-21.

Thrusfield M (2007). Veterinary epidemiology. 3rd ed., Blackwell Publishing.

Workineh SM, Bayleyegne M, Mekonnen H, Potgieter LND (2002). Prevalence and etiology of mastitis in cows from two major Ethiopian dairies. J. Trop. Anim. Health. Prod. 34:19-25.

Zdunczyk S, Zerbe H, Hoedemaker M (2003). Importance of oestrogen and oestrogen active compounds for udder health in cattle: A review. Dtsch. Tierarztl. Wochenschr. 110(11):461-465.

Zerihun T (1996). A study on Bovine sub clinical Mastitis at Stela Dairy farm. DVM Thesis, Addis Ababa University, Faculty of Veterinary Medicine, DebereZeit, Ethiopia. pp. 25–27.

Full Length Research Paper

Study on prevalence and identification of ticks in Humbo district, Southern Nations, Nationalities, and People's Region (SNNPR), Ethiopia

Pawlos Wasihun* and Derese Doda

College of Veterinary Medicine, Haramaya University, Haramaya, Ethiopia, P. O. Box 138, Dire Dawa, Ethiopia.

The distribution and abundance of cattle tick species in Humbo woreda, Wolaita zone, was studied over a period of 6 months from November, 2011 to April, 2012. Adult ticks were collected from seven main body regions of 384 cattle which were under extensive management system. Out of the total of 384 cattle examined, 238 (61%) were found to be infested by one or more tick species. About 2,439 adult ticks were collected from the animal body parts and identified to genera and species level. Five tick species of three genera (*Amblyomma, Boophilus* and *Rhipicephalus*) were identified. The relative prevalence of each species was *Boophilus decolaratus* (30%), *Rhipicephalus evertsi-evertsi* (25%), *Amblyomma varigatum* (25%), *A. cohaerence* (11%), and *A. lepidium* (6%). The risk factors like sex and age of cattle did not show significant association with the infestation rate but there was association with both breeds and body conditions. The prevalence of tick infestation in medium body condition (78%), poor body condition (67%), and good body condition (57%) was found to be statistically significant (p < 0.05) among the three groups of body conditions. The prevalence of tick infestation was found to be statistically significant (p < 0.05) among the three breeds, with highest prevalence in exotic breeds (100%) than both cross (80%) and local breeds (58%). The result indicated that the favorable predilection sites of *Amblyomma* species are ventral body and perineum. *B. decolaratus* preferred dewlap, udder/scrotum, belly, leg/tail, head, and perineum. *R. eversti-evertsi* had a strong predilection sites for perineum, dewlap, udder/scrotum, and ears. The sex ratio of all tick species identified during this study periods was skewed towards male except for *B. decolaratus*. Considering the economic importance of tick and tick borne diseases (TBDs) in the Humbo district, also in the country, there should be country wide control strategy, taking into account acaricide residues in products.

Key words: Attachment site, cattle, Humbo woreda, ixodidae, prevalence, tick burden.

INTRODUCTION

Ethiopia, located in the horn of Africa, between latitude of 30 to 15°N of the equator and longitude 33 to 48°E, is an agrarian country with an estimated total land area of 1,101,000 km². The country has an extremely diverse topography, a wide range of climatic features and multitudes of agro-ecological zonations which makes the country suitable for different agricultural production system. This in turn has contributed to the existence of large diversity of farm animal genetic resources (Annon, 2004). The proportion of total population in agricultural sector is 82.4%. The country has the largest number of livestock in Africa, approximately 44.3 million cattle, 46.9 million sheep and goats, more than 1.0 million camels, 4.5 millions equine, and 40.0 million chickens (Community-supported agriculture (CSA), 2004). Among livestock, cattle play a significant role in socio-economic life of the people of Ethiopia.

Ticks are obligate, blood feeding ecto-parasites of

*Corresponding author. E-mail: pwasihun@yahoo.com.

vertebrates, particularly mammals and birds. It has been estimated that about 80% of the world population of cattles are infested with ticks. The lifecycle of ticks (both Ixodids and Argasids) undergo four stages in their development; eggs, 6-legged larva, 8-legged nymph and adult (Minjauw and McLeod, 2003). According to the numbers of hosts, Ixodids ticks are classified as one-host ticks, two-host ticks, three-host ticks and Argasids classified as multi-host ticks. In one-host ticks, all the parasitic stages (larva, nymph and adult) are on the same hosts; in two- host ticks, larva attach to one host, feed and moult to nymphal stage and engorged, after which they detach and moult on the ground to adult; and in three-host ticks, the larva, nymph and adult attach to different hosts and all detach from the host after engorging, and moult on the ground. In multi-host ticks (Argasids), a large number of hosts are involved and it is common to have five moults, each completed after engorging and detaching from the hosts (Taylor et al., 2007). Ticks are most numerous, particularly in tropical and sub-tropical regions, and their impact on animal health and production is greatest in these regions (Lefebvre et al., 2010). Ticks are usually relatively large and long lived, compared to mites, surviving for up to several years (Kettle, 1995). Ticks belong to the phylum Arthropod, class Arachnid, and order Acari. The families of ticks parasitizing livestock are categorized into two, the Ixodidae (hard ticks) and Argasidae (soft ticks). Though, sharing certain basic properities, they differed in many structures, behavioral, physiological, feeding and reproduction pattern (Urquhart et al., 1996). Ticks that are considered to be most important to domestic animals' health in Africa comprise about seven genera and forty species. Among these tick genera, the main ticks found in Ethiopia are *Ambylomma* (40%)*, Boophilus* (21%)*, Heamaphysalis* (0.5%), *Haylomma* (1.5%), *and Rhipicephalus* (37%) (De Castor, 1997; Minjauw and McLeod, 2003). Among these, *A. varigatum and B. decoloratus* are most important and widely distributed (Abebaw, 2004). *A. coherence, A. gemma, A. lepidium, Haylomma marginatum rufipes, H. truncatum, and R. evertsi* are also commonly found in Ethiopia (Pegram et al., 2004; Solomon and Kaaya, 1996).

Even though there are a number of studies on ticks and TBDs in many parts of Ethiopia, in some location, there was no previous study on tick and TBDs and even other parasitic diseases in Humbo district, Wolaita zone. Therefore, the objectives of this study are to estimate the prevalence of tick infestation of cattle in Humbo district and to identify the common tick species in Humbo district.

MATERIALS AND METHODS

Description of study area

Tick survey was conducted in Wolaita zone, particularly in Humbo

district. The woreda is located 1100 to 2300 meter above sea level, 6° 40'N latitude and 37° 50'E longitude in South Nation Nationalities and People Regional Government (SNNPR), 350 km from capital city of Ethiopia (Figure 1). The climatic condition of the study area was a mean annual temperature of 22.0°C and mean annual rainfall of 1123.15 mm. The woreda has forty kebeles, with total area of 86,646 hectare (ha) which is 70% of lowland and 30% of midland. Out of the total area of the woreda, the agricultural land (38,481 ha), permanent trees (4,980 ha), forest (16.900 ha), grassland (6,581 ha), different sectors (7,194 ha), fertile land (5,140 ha) and marshy area (7,370 ha) were included. It is bordered on the South by West Abaya woreda, North by Sodo zuria woreda, East by Damote Woyde woreda, and West by Ofa woredas. The human population in the area is 152,495; which comprise 75,487 males and 77,008 females. The major crops grown in the study area are cereals such as teff, maize, sorghum, cotton and root crops like sweet potatoes, ensete, carrot and fruits like mango, avocado and banana (HDAB, 2011).

Study population

According to Humbo District Agricultural Beruea (2011), the livestock of Humbo district was cattle (74,713), sheep (13,108), goats (23,209), equine (9,736), and poultry (50,133). The study populations were constituted in all breeds but the mostly populated breed in the area was indigenous or local breeds kept under traditional management system.

Sampling design and sampling technique

A cross sectional study was conducted from November, 2011 to April, 2012 to determine the prevalence of ticks, and identification of species of ticks collected and labeled according to predilection site. All the animals selected as sampling unit were checked for any tick infestation based upon the numbers of ticks found on the animal and the study record period. Ticks were collected from ears, heads, dewlaps, belly/flunk, udder/scrotum, perineum and legs/tails in the separated sample bottles with 70% ethanol. In addition to the attachment site of tick in different body regions, the burden of ticks based on age, sex, body condition, and breeds of animals were determined.

Sampling methods and determination of sampling size

The cattle to be examined were selected by simple random sampling method, and multistage sampling strategy was used to determine appropriate sample size. The sample size was determined by using the formula given in Thrustifield (1995). The expected prevalence of Ixodidae ticks of cattle in Humbo woreda was assumed as 50%. The parameters used were 95% confidence interval and 5% desired level of precision. By substituting these values in the formula, the sample size taken was n = 384

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

Where n = sample size; P_{exp} = expected prevalence; d^2 = expected precision which is usually 5% (0.05).
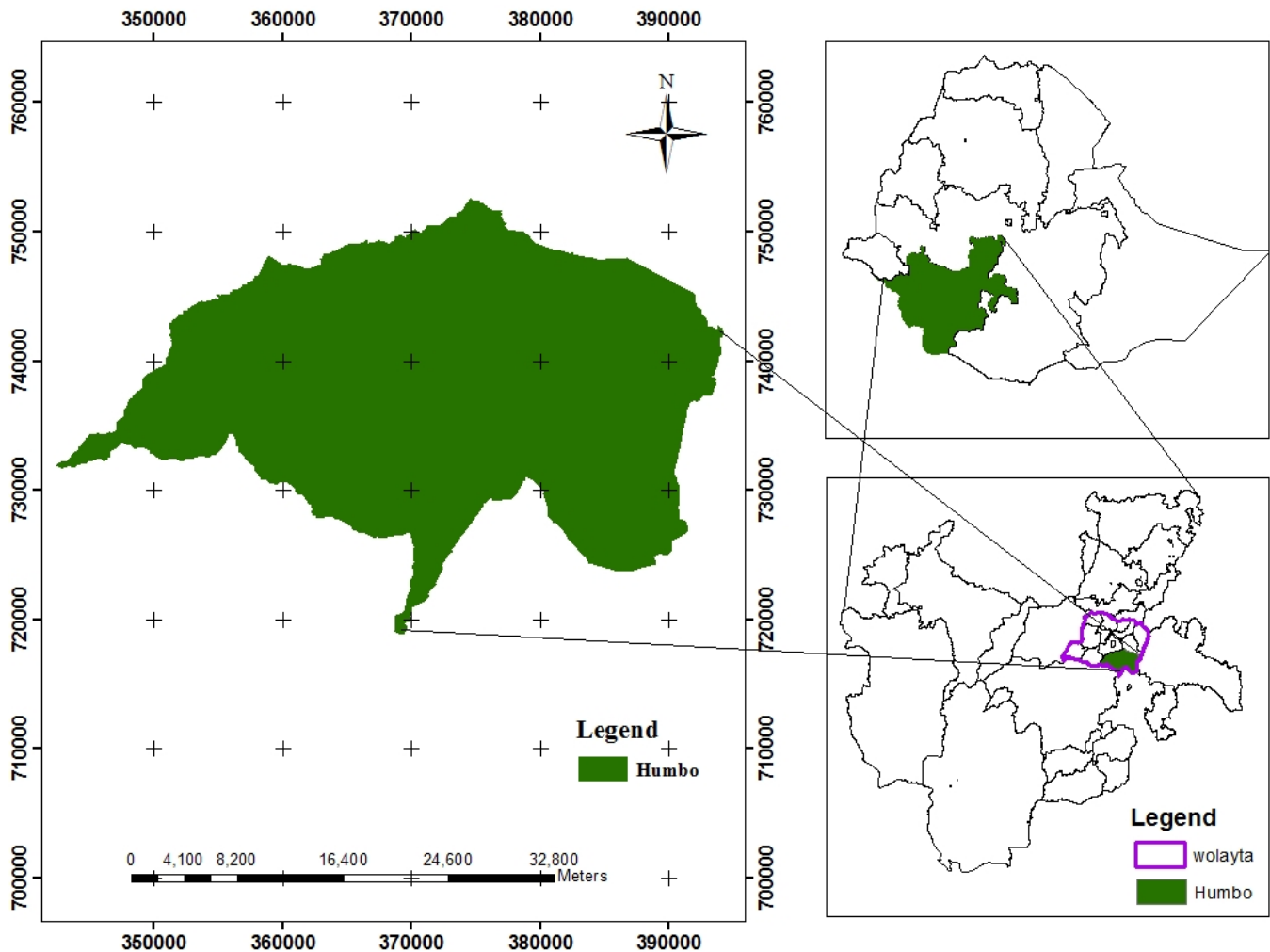
Figure 1. Map of study area.

Study methodology

Firstly, the selected study animal was properly restrained and all tick samples were collected from half the body regions. Ticks were removed carefully and gently in a horizontal pull to the body surface. The collected ticks were preserved in universal bottles containing 70% ethanol and labeled with respect to predilection site, age, sex and date of collection, then transported to Wolaita Sodo Regional Veterinary Laboratory for counting and identification. The ticks were counted and subsequently identified to genus and species level by using stereomicroscope, according to standard identification keys given by Walker et al. (2003).

Data analysis

The data collected were entered and managed in Microsoft-excel. An intercooled STATA 7 version software (Stata Corporation, 2001) statistical program was employed for the data analysis. The overall prevalence of tick was determined by dividing the number of positive animals by total sample size, and was expressed as percentage. Chi-square (x^2) test was used to assess if there was a statistically significant association in tick infestation between ages, sex, breeds and body conditions.

RESULTS

Prevalence of ticks on cattle in Humbo woreda

In this survey, a total of 384 animals where, local (n = 332), cross (n = 41), exotic (n = 11) breeds of cattle were examined. Then the overall prevalence was calculated by dividing the number of positive samples by the total sample size and multiplied by 100. Out of the 384 animals examined, ticks were found on 238 animals yielding an overall prevalence of 61.98%. The distribution of tick genera were identified and located in Table 1. The

statistical analysis was done for the prevalence of tick infestation with hypothesized risk factors (age, sex, breed and body condition).

There were statistically significant association with breeds (x^2 = 14.4791, p = 0.001) and body conditions (x^2 = 19.2801, p = 0.000) (Table 2).

Higher tick infestation rate was seen on both medium body condition and exotic breeds. There were no statistical significances (p > 0.05) associated with sex and age of animals (Table 3).

Identification of tick species and their abundance

Of the total 2,439 Ixodid ticks collected from seven body region of 384 cattles, five different species in three genera were indentified. The tick species identified were *B. decolaratus* (30.63%), *R. evertsi subspecies evertsi* (25.91%), *A. varigatum* (25.42%), *A. cohaerence* (11.36%), and *A. lepidium* (6.68%) in descending order of abundance (Table 5). By considering relative abundance of each tick species identified in the study area, *B. decolaratus* was the most abundant (30.63%) and *A. lepidium* was the least abundant (6.68%).

Predilection site of identified ticks

The observed proportion of tick species attachment site during this study was summarized and shown in Table 4. All three species of *Ambylomma* identified during the study preferred udder/scrotum, dewlap/brisket, perineum, belly/back, legs/tail and head regions. The *B. decolaratus* preferred the attachment site such as dewlap/brisket, belly/back, legs/tail, udder/scrotum, heads, ears, and perineum regions in decreasing order. The *Rhipicephalus* species were encountered mainly in the perineum, dewlap, udder/scrotum, ears, and belly/back and head regions.

Prevalence of tick species in relation to sex of ticks

The numbers of ticks collected from the cattle dominated by males but an exception was found in one host tick (*B. decolaratus)* in which females' collection was higher than the males' (Table 5).

DISCUSSION

The distribution and abundance of tick species infesting cattle in Ethiopia vary greatly from one area to another area. In this study *B. decolaratus* were found to be the most abundant tick species in Humbo district (30.63%).

This is in agreement with Sileshi et al. *(*2007) who described that *B. decolaratus* is the commonest and most wide spread tick in Ethiopia, collected in all administrative regions except in the Afar region. This is also in line with Tamru (2008) in Asela, and Teshome et al. (1995) reported the highest prevalence of *B. decolaratus* (80%) in the study areas. According to Shiferaw (2005) *B. decolaratus* had highest frequency in the observed area during dry seasons (January, February and early March) in Wolaita zone. This result disagreed with the findings of Alekaw (1998) at Metekel Ranch, Ethiopia showing prevalence of 5.7%. This may be due to the geographical location and altitude factors which is 1,500 to 1,600 m above sea level of Metekel Ranch. The females were abundant from September to April and transmitted *Babesia bigemina* to cattle, and severe infestation can lead to tick worry, anorexia and anemia (Seyoum, 2005). The one-host ticks of the genus *Boophilus* that parasitize ruminants represent a hindrance to livestock farming in tropical and sub-tropical countries. They transmit the causative agents of anaplasmosis ("gall sickness") and babesiosis ("red water") in cattle (Walker et al., 2003).

R. evertsi-evertsi was found to be the second most abundant (25.91%) tick species in this study. The native distribution of *R. evertsi-evertsi* in Ethiopia seems to be connected with middle height dry Savannas and steppes, in association with zebra and ruminant and it is widely distributed throughout Ethiopia (Belew and Mekonnen, 2011). This tick species shows no apparent preference for particular altitude, rainfall zone or seasons (Pegram et al., 1981). The result of the current study was in line with Belete (1987) in Nekmet Awarja and Tessema region, and Gashaw (2010) in and around Asela, Hussen (2009) and Belew and Mekonnen (2011). According to Sileshi et al. (2007), *R. evertsi-evertsi* was collected throughout their study period, with the peak of abundance in January coinciding with the beginning of the rainy season and they also described that the discovery of this tick in that area was in line with its widespread occurrence in most parts of the country. The occurrence of this species in and around Wolaita zone was also reported by Dessie and Getachew (2006). *R. evertsi-evertsi* has short mouth parts with which to feed on soft area. As a result, it is a possible vector of *Babesia, Rickettsia* and *Theleria* (Kettle, 1995).*A. varigatum* was the third widespread tick species of the cattle in the current study area (25.42%). This result disagreed with different reports done by other authors in different parts of Ethiopia such as Tessema and Gashaw (2010) in Asela, Belew and Mekonnen (2011) in Holeta, Seyoum (2005), Mehair (2004) in Awassa who as described *A. varigatum* as the first most abundant tick species in their study areas. The difference in result was due to the geographical location where *A. varigatum* was found in highest number in the highland and high rainfall, and also due to its being relatively

Table 1. Distribution of tick genera of cattle in the study area.

Kebeles	Tick genera							
	Amblyoma		*Boophilus*		*Rhipicephalus*		Total	
	No.	%	No.	%	No.	%	No.	%
Abalala Faracho	239	54.07	98	22.2	105	23.76	442	18.12
Koysha Ogodama	114	39.18	103	35.4	74	25.43	291	11.92
Shocora Pisho	242	36.8	266	40.5	149	22.68	657	26.94
Demba Koysha	180	35.8	155	30.8	168	33.4	503	20.62
Humbo kebele 01	212	63.5	46	13.8	76	22.75	334	13.7
Humbo kebele 02	73	34.4	79	37.3	60	28.3	212	8.7
Total	1,060	43.46	747	30.63	632	25.91	2,439	100.00

Table 2. Prevalence of tick in relation to body conditions and breeds of animals.

Parameter	Body condition			Breed		
	Good	Medium	Poor	Local	Cross	exotic
No. of animal examined	239	89	56	332	41	11
Infested animals	129	71	38	194	33	11
Prevalence (%)	58.3	79.78	67.86	58.43	80.49	100

Body condition: $X^2 = 19.2801$, p = 0.000; Breed: $X^2 = 14.4791$, p = 0.001.

Table 3. Prevalence of tick in relation to body conditions and breeds of animals.

Parameter	Sex		Age	
	Male	Female	≤5 years	>5 years
No. of animal examined	213	171	144	240
Infested animals	134	104	87	151
Prevalence (%)	62.91	60.82	60.42	62.92

Sex : $X^2 = 0.1762$, p = 0.675; Age: $X^2 = 0.2387$, p = 0.625.

Table 4. Numbers of tick species identified in half body region of cattle.

Tick species	Body regions						
	Head	Ear	Dewlap	Legs/tail	Udder/scrotum	Perineum	Belly
A.varigatum	6	-	290	6	263	53	2
A.cohaerence	-	-	9	-	153	25	17
A.lepidium	-	-	58	-	95	10	-
B.decolaratus	24	17	450	74	66	16	100
R.evertsi-evertsi	2	31	112	-	55	425	7
Total	32	48	919	80	632	529	126

active throughout the year in most part of Ethiopia. *A. varigatum* is a widely distributed cattle tick in Ethiopia (Morel, 1980), and it is a potential vector of diseases caused by *Cowdria ruminantium, Theleria mutan, T.*

Table 5. The distribution and sex ration of adult tick species in the study area.

Tick species	Total count	Prevalence (%)	Sex		Ratio
			Male	female	(male: female)
B. decolaratus	747	30.63	5	742	0.007:1
R.evertsi-evertsi	632	25.91	563	69	8.2:1
A.varigatum	620	25.42	532	88	6.1:1
A.cohaerence	277	11.36	251	26	9.7:1
A.lepidium	163	6.68	137	26	5.3:1
Total	2,439	100.00	1448	951	1.5:1

velifera ("benign bovine thelieriosis") and viral diseases, Nairob sheep disease, and also aggravates the situation of bovine dermatophilosis *(Dermtophilus congolence)* (Sileshi et al., 2007).

The study conducted in Wolaita zone by Dessie and Getachew (2006) showed that *A. varigatum* was the second abundant tick species at highland and midland, and the first abundant in the lowland during wet period. This variation may be due to the change in environmental conditions, with the result of global warming that highly affect the ecology of ticks. Change in temperature and rainfall have been reported to affect the distribution of diseases of vectors and tick borne diseases (Taylor et al., 2007).

A. cohaerence was the fourth abundant tick species (11.36%) in the study area. According to Dessie and Getachew (2006) *A. cohaerence* was also the fourth abundant tick species in the Wolaita zone with significant seasonal distribution variation at highland and lowland. This result was also similar with the reports of Belew and Mekonnen (2011) in Holeta, and Tessema and Gashaw (2010) in Asela. In tick survey conducted in western Ethiopia, *A. cohaerence* was found to be the most prevalent in Mezan Teferi (Seid, 2004) and Jimma (Yitbarek, 2004), with the prevalence of 50.5 and 83.1%, respectively. The prevalence of *A. cohaerence* is alarmingly important as this tick has been reported as a vector for *C. ruminantium* which is the causative agent of cowdriosis ("heart water") (Sileshi et al., 2007).

A. lepidium was the least abundant tick species in the study area (6.68%). It is limited by semi-desert conditions (Morel, 1980). It is also known as the "East African bont tick" and is common in many of the semi-arid regions of East Africa (Walker et al., 2003). *A. lepidium* was common but not abundant in Wolaita zone according to Dessie and Getachew (2006) which is similar with the present finding that *A. lepidium* was the least abundant in the area. This is also similar with the findings of Mesele et al. (2010) in Bedelle district. In southwest of Ethiopia including Gambella region and western Oromiya, this tick species was also reported with less abundance by

several workers (Mekonnen, 1995; Pegram et al., 1981; De Castor, 1994) that agree with the current result.In Gambella region, *A. lepidium* transmits *C. ruminantium*, *Rickettsial* organism that causes cowdriosis. *A. lepidium* was irregularly dispersed throughout most of the country and was collected from Tigray, Amhara, Oromiya, SNNP and Harare RegionalStates (Sileshi et al., 2007). Ticks are known to be distributed in different parts of the host's body. In this study, the main infestation site of ticks in the body of hosts was dewlap, udder/scrotum, perineum, and belly. A variety of factors such as host density, interaction between tick species, time and season, and inaccessibility for grooming determined the attachment site of the ticks on the skins (Solomon et al., 2001). The predilection sites found in this study were in line with those reported by Seyoum (2001) and Behailu (2004) in their study conducted in North Wollo zone and Asela, respectively.

In this study, different animal related risk factors were studied to determine whether there is a significant variation in tick infestation between and among different groups of animals with suspected risk factors. The proportion of tick infestation was higher in adult animals as compared to young animals. However, there was no statistically significant association (p > 0.05), and the higher proportion may be due to outdoor management and long distant movement of adult animals to search for food and water compared to younger animals, so the chance of exposure is higher. This finding is also in agreement with the finding of Feseha (1997), Tessema and Gashaw (2010) and Belew and Mekonnen (2011) who stated a higher proportion in adult cattle.There was also statistically non-significant association (p > 0.05) in the infestation rate among different sex groups, where higher infestation was recorded in male animals compared to their counter parts. This variation may be associated with female animals which were kept properly in the house with good management system for dairy purpose whereas male animals grazing on field all day may be exposed to tick infestation. This result also agreed with the previous work done by other author

(Hussen, 2009) in Bako.

The proportion of tick infestation was higher in medium body conditioned (79.78%) as compared to poor body conditioned (67.86%) and good body conditioned animals (58%). This was due to the fact that medium body scored animals have reduced resistance and are exposed to any kind of disease when grazing on the field, and poor body conditioned animals were kept at home due to their inability to walk long distant areas, so they become less infested than medium sized animals but the well fed animals were very resistant to any kind of diseases when they grazed in the field or are kept at home.The fact that more tick burden was recorded in both exotic and cross breeds compared to local cattle would help in planning for tick control before the introduction of different improved breeds. In the past, attempts to introduce cattle of exotic inheritance into tropics have not had expected success. One reason for the failure could be because of the high susceptibility to ticks and TBDs of exotic and cross breeds. However, the fear of introducing susceptible cattle can be solved by introducing certain degree of resistance in these cattle by means of prophylactic treatment before introducing into enzootic areas (Solomon et al., 2001). This result is in agreement with Tessema and Gashaw (2010). In contrast, the report by Belew and Mekonnen (2011) revealed that the presence of tick infestation in local breeds were very high with the prevalence of 44.96% (n = 223), while in cross breeds and Jersey, the prevalence were 15.83% (n = 57), and 8.50% (n = 30), respectively. The significant variation in tick infestation of cattle of different breeds in their research might be attributed to different management system, lack of supplementary feeding for local breeds, or lack of control measures against tick on local breeds. Furthermore, it can be assumed that it might be due to lack of interest of farmers for local breeds as well as taking more care to cross and exotic breeds than local breeds.

The male to female rations of *B. decolaratus, R. evertsi-evertsi, A. varigatum, A. cohaerence* and *A. lepidium* were similar to previous reports (Seyoum, 2001; Solomon et al., 2001). Except *B. decolaratus*, all other species tick's males outnumbered females because males normally remain on the host longer than females. Fully engorged female tick drops off to the ground to lay eggs while male tend to remain on the host up to several months to continue feeding and mating with other females on the host before dropping off (Solomon et al., 2001). The females of *B. decolaratus* outnumbered males in this study probably due to small size of male which may not be seen during collection (Tessema and Gashaw, 2010).

Acaricide usage is still the main choice of tick control in the area. Currently organophosphate acaricides are most widely used chemicals. However, organophosphate resistance is emerging, especially in one-host ticks. Tick control can be also achieved by attacking one or more larval phase along the life cycle chain (Food and Agriculture Organization (FAO), 1984). In addition to acaricide application, appropriate livestock management, zero-grazing, up-grading of tick resistant cattle and implement traditional practices are quite important.Based on the information gathered during the study period from various cattle owners, infestation rate and tick burden increases after short rainy season (May to June) and decreased during long dry season (January to March). It is possible to indicate the trend of seasonality of tick population by comparing the number of ticks collected in the study period, there was a change in number of ticks from slightly wet months to the dry months, similar result were reported by Solomon et al. (2001), Tessema and Gashaw (2010) and Hussen (2009).

CONCLUSION AND RECOMMENDATIONS

Variable information on tick species distribution and dynamics are very essential to assess the economic loss encountered due to tick infestation and also to identify the appropriate measure of tick control. Among ecto-parasites, ticks cause the greatest economic loss in livestock population either by transmitting a wide variety of TBDs or by affecting the health of animals as well as the quality of hide and skins. The important and abundant tick species investigated in the study area were *B. decolaratus, R. evertsi-evertsi, A. varigatum, A. cohaerence,* and *A. lepidium*. The study indicated that there was high burden of ticks in the area. However, the attention given to controlling the infestation had not been sufficient. The control methods necessary for tick and TBDs were selection of tick resistance cattle, acaricides treatment, appropriate livestock management, evaluation and incorporation of traditional practices or remedies that appear to be of value.

In general, the distribution of ticks are not fixed but are determined by a complex interaction of factors such as climate, host density, host susceptibility, grazing habits, and pasture-herd management. Therefore, effective tick control program should be formulated and implemented based on the distribution pattern of ticks and factors responsible for their distribution. In light of the above conclusion the following recommendations are forwarded:

1. Tick control program (application of acaricides) should be continued with an increasing frequency of application in wet months.
2. Detection of acaricide resistance tick species which are economically important since limited types of acaricides were used in the area.
3. More attention should be given to the selection of

resistance cattle breeds and types, and good performance with regards to production of local breeds.
4. Appropriate pasture management in communal grazing area is important.

REFERENCES

Abebaw G (2004). Seasonal dynamics and host preference of *Boophilus decolaratus* on naturally infested cattle in Jimma zone, South Western Ethiopia. Ethiopia Vet. J. 18(1):19-28.

Alekaw S (1998). Distribution of tick and tick borne diseases at Metekel Ranch. Ethiopia Vet. J. 4(1):30.

Annon (2004). State of Ethiopia's Animal's genetic resources country report. Contribution to the first report on the state of the World's animal genetic resources. Institute of Biodiversity Conservation (IBC), Addis Ababa, Ethiopia p. 74.

Behailu A (2004). A survey of ticks and TBDs in cattle in Arsi zone. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Ethiopia.

Belete M (1987). A preliminary survey of ticks on four domestic animals in NekmetAwarja. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeite, Ethiopia.

Belew T, Mekonnen A, (2011). Distribution of Ixodidae ticks on cattle in and around Holeta Town, Ethiopia. Global veterinaria 7(6):527-531.

Central Statistics Authority CSA (2004). Ethiopia agricultural sample enumeration result for SNNPR. Statistical report on livestock and farm implements. Part IV:29-304.

De Castro JJ (1994). Survey of tick species in Western Ethiopia including the previous finding and recommendation, for further tick survey in Ethiopia. Technical report AGDT/ETH/83/023, FAO, Rome pp. 1-83.

De Castro JJ (1997). Sustainable tick borne diseases control in Western Ethiopia. J. S. Afr. Vet. Assoc. 71(4):240-243.

Dessie S, Getachew A, (2006). Cattle tick dynamics in different agro-ecological zones of Wolaita, Southern Ethiopia. Ethiopia Vet. J. 10(2):85-99.

FAO (1984). Ticks and TBDs control. A practical field manual volumem1 tick control. FAO, Rome pp. 1-299.

Feseha B (1997). Species composition and distribution of *Ixodid* ticks in Eastern Harerghiea, Ethiopia. Agr. Sci. 16:37-51.

Humbo District Agricultural Beruea, HDAB (2011).

Hussen Y (2009). Preliminary survey of cattle tick species and burden in and around Bako Town. DVM Thesis, school of Veterinary Medicine Jimma University, Jimma, Ethiopia.

Kettle DS (1995). Medical and Veterinary Entomology. 2nd Ed. UK: CAB international pp. 420-460.

Lefebvre PC, Blancou J, Chermette R, Uilenberg G (2010). Infectious and Parasitic diseases of livestock. 1:93-128.

Mehari B (2004). Distribution of livestock tick species in Awassa Area. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeite, Ethiopia.

Mesele A, Mohammed T, Abebe R, Aragaw K, Bekele J (2010).Survey of *Ixodids* ticks in Domestic ruminants in Bedelle district, south western Ethiopia. Trop.Anim.Health prod. 42:1677-1683.

Minjauw B, McLeod A (2003). Tick borne diseases and poverty. The impact of ticks and tick borne diseases on livestock owners in India and Eastern health program center for tropical veterinary medicine, University of Edinburgh, UK pp. 24-57.

Morel P (1980). Study on Ethiopian ticks (*Acaridae: Ixodidae*). 1st.Ed. Republic of France, Ministry of Foreign affairs, French Vet. Mission, Addis Ababa, Ethiopia pp. 15-183.

Pegram PG, Tatchell RJ, De Castro JJ, Chizyuka MJ, Mc Cosker PG, Moran MC, Nigarura G (2004). Tick control: New concepts, www.fao.org/AGA/AGAP/FRG/Feedback.htm.

Pegram RG, Hoogstral, Wassef HY (1981). Ticks (Acari: *Ixodidae*) of Ethiopia. Distribution, ecology, and relation of species infesting livestock. Bull. Entomol. Res. 71:335-359.

Seid B (2004).Survey of cattle tick species in and around Mezan Teferi, Bench Maji zone of SNNPR.DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeite, Ethiopia.

Seyoum Z (2001). Study of tick and TBDs on cattle at Girran valley in the North Wollo Zone. Proceeding the Ethiopia Vet. Assoc. p. 15.

Seyoum Z (2005). Distribution and host parasite relationship of *Ixodids* ticks in Eastern Amhra, Kombolcha Regional Veterinary Laboratory, Kombolcha, Ethiopia pp. 34-47.

Shiferaw D (2005). Cattle tick dynamics in different agro-ecological zones of Wolaita, Southern Ethiopia. Master degree thesis. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeite, Ethiopia pp. 1-137.

Sileshi M, Pegram RG, Solomon G, Abebe M, Yilma J, Sileshi Z (2007). A synthesis of review of *Ixodid*s (*Acari: Ixodidae*) and *Arga*s (*Acari: Argasidae*) ticks in Ethiopia and their possible role in diseases transmission. Ethiopia Vet. J. 2:1-22.

Solomon G, Kaaya GP (1996). Studies on the developmental and survival of *Rhipicephalus pulchelu* and *Amblyoma gemma* under field condition. Ethiopia Vet. Assoc.

Solomon G, Night M, Kaaya GP (2001). Seasonal variation of ticks on calves at Sabetha, Western Shewa zone. Ethiopia Vet. J. 7(1&2):17-30.

STATA Corporation (2001). Stata statistical software Release 7.0. College station, Texas.

Tamru T (2008). Survey of Bovine tick species in and around Asela Town, DVM Thesis, School of Veterinary Medicine, Jimma University, Jimma, Ethiopia.

Taylor MA, Coop RH, Wall RL (2007). Veterinary Parasitology.3rd Ed. Black Well Publishing, London pp. 679-712.

Teshome Y, Feseha G, Wakjira A, Tsega T (1995). Preliminary observation on ticks: Seasonal dynamics and resistance of three indigenous and three cross breed cattle in Ethiopia. Bull. Anim. Health. Prod. Afr. 43:105-114.

Tessema T, Gashaw A (2010). Prevalence of ticks on local and crossbreed cattle in and around Asela Town, South East, Ethiopia, Amber Animal Health Department, East Gojam, Ethiopia Vet. J. 14(2):79-89.

Thrustfield M (1995). Veterinary Epidemiology. 2nd Ed. UK Black Well Science Ltd., pp. 182-198.

Urquhart GM, Armour J, Dunean JI, Dunn AM, Jennings FW (1996).Veterinary parasitology. 2nd Ed. Black Well Science. The University of Glaskow, Scotland, UK pp. 181-188.

Walker AA, Bouatour A, Camicas JL, Estadapena AA, Harok IG, Hatif AA, Pegram RG, Preton PM (2003). Ticks of domestic animals in Africa: A guide to identification species. The University of Edinburgh, UK pp. 67-80.

Yitbarek G (2004). Tick species infesting livestock in Jimma, Southern Western Ethiopia, DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeite, Ethiopia.

Full Length Research Paper

Camel brucellosis and management practices in Jijiga and Babile districts, Eastern Ethiopia

Berhanu Tilahun[1]*, Merga Bekana[2], Kelay Belihu[2] and Endrias Zewdu[3]

[1]College of Veterinary Medicine, Haromaya University, P. O. Box 138, Dire-Dawa, Ethiopia.
[2]Faculty of Veterinary Medicine, Addis Ababa University, P. O. Box 34, Debre-Zeit, Ethiopia.
[3]Faculty of Agriculture and Veterinary Sciences, Ambo University, P. O. Box 19, Ambo, Ethiopia.

A cross-sectional study was carried out on sera of 822 randomly selected camels in order to estimate seroprevalence and risk factors of brucellosis and assess camel management practices. A questionnaire survey was administered to one-hundred willing respondents out of the total 185 camel owners whose camels were included in the sample unit. The sera were first screened by Rose Bengal plate test (RBPT) and then all positive reactors were further tested by the complement fixation test (CFT) for confirmation. The overall seroprevalence of brucella in camels was 2.43% (95% CI = 1.3 - 3.8). None of the potential risk factors studied (district, sex, age, herd size, camel rearing experience and parity) had significant effect on animal level seroprevalence (P > 0.05). The herd level seroprevalence was significantly associated with abortion (P = 0.012) and still birth (P = 0.016). Significant proportion (40%) of camel herders kept camels together with cattle, sheep and goats. Thirty-two percent of camel herders kept camel with cattle. The camel herd composition was dominated by pregnant (21.8%), lactating (21.1%) and mature non-lactating she camels (19.3%). The major diseases affecting camels were trypanosomiasis (93%), anthrax (80%), pneumonia (70%), "bent neck" (59%), abscess (59%), endoparasites (54%) and ectoparasites (51%). Camel management practices like herding, watering, milking, delivery and mating assistance were mainly the responsibilities of adults and young males. Although, seroprevalence of camel brucellosis was low, it could pose considerable threat to public health and market value of camels. The camel health and management practices are inadequate. Public education and detailed epidemiological studies of camel diseases were suggested.

Key words: Camel, brucellosis, seroprevalence, milking, watering, herding, risk factor.

INTRODUCTION

Camels play an important socio-economic role within the pastoral and agricultural system in dry and semi dry zones of Asia and Africa (Gwida et al., 2011). Camels are known to have peculiar physiological features by which they regulate body temperature to changes in ambient temperatures, enabling them to survive and produce under harsh environmental conditions. These characteristics features of camels have made it possible to use to use marginal and desertified ecosystems and over the

centuries, the camel has been a symbol of stability for the pastoralists in the arid zones of the world (Yagil, 1985; Higgins et al., 1992; Abbas et al., 1992).

Like other livestock or even more, camels are susceptible to common diseases including brucellosis (Wilson 1984; Abbas and Tilley, 1990). Brucellosis is an infectious disease of animals and humans caused by a number of host-adapted species of genus *Brucella* (Radostits et al., 2006; Mantur et al., 2007). The disease in animals is characterized by abortions or reproductive failure (Abbas and Agab, 2002). Camels are highly susceptible to brucellosis caused by *Brucella melitensis* and *Brucella abortus* (Abbas and Agab, 2002; Gwida et al., 2011) especially when they are pastured together with

---
*Corresponding author: E-mail: berhanutilahun@ymail.com. Tel: +251 911011881. Fax: +251 255530325.

infected sheep, goats and cattle. The large herd size, sharing of watering points with ruminants and inadequate hygienic practices under pastoral management system favors transmission of camel brucellosis, particularly at time of abortion or delivery, by an infected female (Abbas and Agab, 2002).

Brucellosis is transmitted to humans mainly by direct contact with infected livestock and the consumption of unpasteurized contaminated milk and dairy products (Musa et al., 2008). Cattle, goat, sheep, camels and other livestock may be infected and transmit the disease to human populations. Pastoralists in endemic areas are at high risk of infection by *Brucella* species (Skalsky et al., 2008).

In Ethiopia, camels are a subset of large livestock resource with a population of 2.3 million (CSAE, 2004). Among the pastoral and agropastoral communities of Ethiopia, camels are the most important livestock species uniquely adapted to live in hot and arid environments that are inhospitable to other domestic animals. Camels are traditionally raised by these communities primarily for milk production (Demeke and Kumsa, 1997). Despite the presence of large population of camel in the pastoral areas of Ethiopia, reports of camel brucellosis (Dominech, 1977; Richared, 1980; Teshome et al., 2003; Megersa et al., 2005) and studies of management practices are limited; in particular no published information is available for camel brucellosis in Babile district.

Seroprevalence of camel brucellosis in Jijiga district was earlier reported by Teshome et al. (2003). The aims of the present study were to estimate the seroprevalence of camel brucellosis, identify potential risk factors to acquire the disease and assess camel management practices in Jijiga and Babile districts of Jijiga zone, Eastern Ethiopia.

MATERIALS AND METHODS

Study area

A cross-sectional study was carried out from October, 2005 to March, 2006 in Jijiga and Baile districts of Jijiga Zone. Jijiga district is located 9° 35' N latitude and 42° 8' E longitude and has an elevation of 1,609 m above sea level (masl) (htpp//populationmongabay.com/). The climate is generally semiarid and arid with 402.9 mm annual average rainfall. The annual daily minimum and maximum temperature ranges from 12.8 to 28.3°C (NMAEJB, 2006). Babile district is located 8°40' 0" N latitude and 42° 25' 0" E longitude and has an altitude ranging from 950 to 2000 masl (htpp// population mongabay.com/). The districts are inhabited by different tribes of Somali communities of which the Yebere, Abskul, Gedebursi, Malingur, Bertire, Giri, Hawya and Jarso are known camel rearing tribes.

Study design, study animals and blood collection

A cross-sectional study was carried out on 822 selected camels of both sexes with no history of vaccination against brucellosis. Sample size was determined according to Thrusfield (2005) for

random sampling and calculated using the expected prevalence of 4.16% (Teshome et al., 2003), 95% confidence interval and 2% absolute precision. The minimum sample size calculated was 382 however; it was inflated to 822 for better precision. Babile and Jijiga districts were purposively selected based on their accessibility and camel population. Then, 36 settlements (kebeles) were randomly selected from both districts. Camel populations found in these settlements were the study population where individual animals were sampled using systematic random sampling. Camels aged two and above years were included in the study. Herd consisting ≥ 35 and ≤ 34 camels were considered as large and small herds, respectively. Blood samples were collected from the jugular vein using plain vacutainer tubes. The samples were left at room temperature overnight to allow clotting for sera separation. The separated sera were stored at -20°C until serologically tested.

Rose Bengal plate test (RBPT)

All collected sera were initially screened for antibodies against *Brucella* by the Rose Bengal plate test (RBPT). The test was performed using commercially available antigen (Institute Pourquer, 3409 Montpellier Cedex 5, France) following the method described by Alton et al. (1975) and OIE (2004).

Complement fixation test (CFT)

All sera reacted positive to the RBPT were further tested using CFT for confirmation. The CFT was performed at the National Veterinary Institute in DebreZeit, Ethiopia, using the protocols recommended by OIE (2004). A standard *B. abortus* antigen for CFT (Veterinary Laboratories Agency, United Kingdom) was employed to detect the presence of antibodies against *Brucella* in the sera. The control sera and complement were both obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany. Sera with a strong reaction that is more than 75% fixation of the complement (3+) at a dilution of 1:5 and with at least 50% fixation of the complement (2+) at dilutions of 1:10 and 1:20 were classified as positive (+).

Questionnaire survey

A questionnaire survey was administered to one-hundred willing respondents out of the total 185 camel owners whose camels were included in the sample unit. The information gathered relates to livestock structure, composition of camel herds, camel rearing experience, camel management (milking, herding, watering, delivery and mating assistance), milk consumption habits and purpose of camel rearing. Additionally, age, sex, herd size, parity and physiological status of sampled camels were recorded.

Data analysis

The data generated were stored in Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for windows (Stata Corp. College Station, USA). Variables with more than two categories were transformed into indicator (dummy) variables. Herds containing at least one seropositive camel were considered positive. Seroprevalence was calculated by dividing the number of camel tested positive (CFT) by the total number of camels tested. Similarly, herd-level seroprevalence was calculated as the number of herds with at least one positive camel divided by the total number of herds tested. Association between the occurrence of *Brucella* infection and the potential risk factors on

Table 1. Results of serological diagnosis of camel brucellosis by RBPT and CFT in Jijiga and Babile districts of Somali region.

Location	N	RBPT		CFT	
		No. positive	%	No. positive	%
Jijiga	594	23	3.18	17	2.86
Babile	228	5	2.19	3	1.32
Total	822	28	3.41	20	2.43

N = number of camels examined; No. = number.

Table 2. Univariate logistic regression analysis of potential risk factors associated with animal level camel brucellosis.

Risk factor	Category	Tested	No. positive	Prevalence (%)	Univariate	
					OR (95% CI)	P-value
District	Babile	228	3	1.32	1.0	
	Jijiga	594	17	2.86	2.21(0.64 - 7.61)	0.209
Sex	Female	641	15	2.34	1.0	
	Male	181	5	2.76	1.19(0.43 - 3.31)	0.745
Age	≤ 4 years	174	3	1.72	1.0	
	5 - 10 years	449	10	2.23	1.30(0.35 - 4.77)	0.694
	≥ 11 yrs	199	7	3.52	2.08(0.53 - 8.16)	0.295
Herd size	Small	573	13	2.27	1.0	
	Large	249	7	2.81	1.25(0.49 - 3.16)	0.643
Camel rearing experience	≤ 30 years	463	9	1.94	1.0	
	≥ 31 years	359	11	3.06	1.59(0.65 - 3.89)	0.305
Parity	Zero	188	2	1.06	1.0	
	First	158	5	3.16	3.04(0.58 - 15.88)	0.188
	Second	64	3	4.69	4.57(0.75 - 8.02)	0.100
	Third	231	5	2.16	2.06(0.39 - 10.73)	0.392
Abortion	No	584	13	2.23	1.0	
	Yes	57	2	3.51	1.60(0.35 - 7.26)	0.544

No. Pos. = number positive, exp = experience, OR = odds ratio, CI = confidence interval.

both herd and animal level were studied using logistic regression. Non-collinear variables that presented P-value of < 0.25 in univariable analysis were offered to the multivariable regression model. For statistical inference, the level of significance was set as 0.05.

RESULTS

Seroprevalence of camel brucellosis

The overall animal level seroprevalence of camel brucellosis was 2.43% (95% confidence interval (CI) = 1.38 to 3.49). The antibody titers ranged from 1:10 to 1:320. Among the 36 settlement areas included in the study, brucellosis was detected in 11 (30.6%) dispersedly located settlement areas. Higher seroprevalence was found in Jijiga district than in Babile (Table 1).

Results of univariate logistic regression analysis of potential risk factors at animal level revealed that all the variables investigated had no significant association with *Brucella* seropositivity (P > 0.05). High seroprevalence was observed in camels older than 11 years of age (3.52%) than in those under 4 years of age (1.72%). The seroprevalence was also higher in male (2.76%) animals than in females (2.34%) and the seroprevalence increased with parity number and herd size (Table 2). Similarly, none of the variable offered to the final model were significant predictors of camel brucellosis (Table 3).

Out of the 185 herds investigated, 28 (15.14%) and 19 (10.27 %) herds reacted positively for RBPT and CFT,

Table 3. Multivariable logistic regression model for predictors' of animal level camel brucellosis.

Risk factor	Category	Adjusted odds ratio (95% CI)	P-value
District	Babile	1.0	-
	Jijiga	2.12 (0.61 - 7.37)	0.238
Parity	None	1.0	-
	One	2.98 (0.57 - 15.61)	0.196
	Two	4.40 (0.72 - 27.04)	0.109
	Three	2.04 (0.39 - 10.65)	0.397

Table 4. Logistic regression analysis of potential risk factors associated with herd level camel brucellosis in Jijiga and Babile districts.

Risk factor		Herd tested	Positive	Prevalence (%)	Univariate		Multivariate	
					Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
District	Babile	52	3	5.77	1.0	-	1.0	-
	Jijiga	133	16	12.03	2.23(0.62, 8.01)	0.218	3.70(0.88, 15.60)	0.075
Herd size	Small	148	13	8.78	1.0	-	1.0	-
	Large	37	6	16.22	2.01(0.71, 5.70)	0.190	1.07(0.33, 3.48)	0.911
Abortion	No	128	8	6.25	1.0	-	1.0	-
	Yes	57	11	19.30	3.59(1.36, 9.48)	0.010	3.91(1.36, 11.25)	0.012
Still birth	No	159	13	8.18	1.0	-	1.0	-
	Yes	26	6	23.08	3.37(1.15, 9.86)	0.027	4.35(1.31, 14.42)	0.016

respectively. Within herd, seroprevalence varied from absence of reactor animals to presence of two reactors out of the herd (0 to 7.7%). As observed in the current study, abortion and still birth had a significant association with herd level seroprevalence both by univariate and multivariate logistic regression analysis ($P \leq 0.05$). Although statistically not significant, higher sero-prevalence was seen in large (16.22%) than in small herd size (8.78%) during herd level analysis (Table 4).

Questionnaire survey

The questionnaire survey revealed that extensive management system was exercised in the area; camels are kept alone as well as together with other species of animals mainly for milk production, and other functions including transport and social security. The highest proportion (40%) of the camel herds kept together with cattle, sheep and goats, while 32% of camel herds were kept only with cattle, 8% with sheep and goats, 4% with cattle and equine and 16% camel herds alone. The mean

camel herd size was 21.7 with the maximum and minimum values being 100 and 4, respectively. The camel herd composition was dominated by pregnant camels (21.8%) followed by lactating (21%) and non-lactating camels (19.3%). Camel bulls constituted only 12.4% of the herd. Females in general make up about 74.6% of the total herd while immature camel made 25.4% of the herd. The camel rearing experience of pastoralists ranged from 4 to 50 years with a mean of 23.97 years. In the present study, it was observed that pastoralists mainly keep camels for milk production (84%). Other purposes of keeping camels include draught mitigation (10%) and herd accumulation (6%). Cattle were mainly kept for milk production while sheep and goats were used as the sources of meat for home consumption and immediate cash income following sale. Donkeys were kept for transportation of water and other goods for home usage.

According to the respondents, 75% of the total milk production was sold to the nearby urban dwellers (mainly in Jijiga town) to generate income. The remaining 25% milk was used for home consumption. All the herders

(100%) consumed fresh raw milk without any heat treatment. They also consume milk after mixing with boiled tea. Camel meat was consumed in the area cooked; however, 18% of respondents consume liver and hump of camel as raw. In the family, activities like herding and watering were done by young and adult males but milking of camels was done mainly by adult males (56%), followed by young males (31%) and females (13%).

Camel owners use traditional wells (59%) and ponds (41%) as the main water sources during dry season for their camels. Camels stay without drinking water for 5 to 20 days in the dry season and for more than 24 days during wet season, due to the fact that they also get water indirectly from the green feed available (mainly cactus). Camels were allowed to drink water from rivers in 20 to 24 days interval. Rivers found in Jerer, Fafen and Daketa valleys are used as a source of water for camels during rainy season. Camels move about 30 to 40 km/s in search of water and pasture during draught period. Prevalent camel diseases reported by respondents include trypanosomosis (93%), anthrax (80%), pneumonia (70%), "bent neck" (59%), abscess (59%), endoparasites (54%), ectoparasites (51%), abortion (30%), wound (23%) and paralysis (5%). Furthermore, trypanosomosis (54%), anthrax (20%), endoparasites (9%), toxic plants (8%), sunstroke (5%) and pneumonia (4%) were mentioned as causes of abortion in camels.

Delivery and mating assistance to camels were strictly the job of adult males (99%) and young males had very limited role in these aspects (1%). Most of the camel owners (65%) receive animal health service from public veterinary clinics. However, 30% of respondents administered drugs to their camels by themselves while 5% were dependent on traditional healers. It was reported that symptoms suggestive of brucellosis including abortion, stillbirth, and swollen joints occurred in 64, 35 and 27% of camel herds in a year, respectively. Usually, aborted camels are removed from the herd mainly by means of selling. Aborted fetus, placenta and discharges were left on the ground. Most of the herders used breeding bull from their own herd (90%) while (10%) used communal village bull. The majority of camel owners (61%) allow camels to graze separately while (34%) practiced grazing of camels with other species of animals. Ninty-eight percent of camel herds had separate night resting area and 2% of camel herds shared night enclosures with cattle and small ruminants.

DISCUSSION

The present study revealed 2.43% overall seroprevalence of camel brucellosis. This seroprevalence is in agreement with the previous reports of 2.8% by Teshome et al. (2003) and 1.8% Megersa et al. (2005) from Ethiopia, 3.1% Omer et al. (2000) from Eritrea, 0.3 to 1.9% Baumann and Zessin (1992) and 3.1% Gahanem

et al. (2009) from Somalia. However, relatively higher seroprevalence of camel brucellosis has been recorded in Jordan 19.4% (Dawood, 2008), in Sudan 30.5% (Omer et al., 2007), in Darfur (Western Sudan) 23.8% (Musa et al., 2008) and in Egypt 7.3% (El-Boshy et al., 2009). The low seroprevalence observed in the present study might be due to the low density of camel population kept in a widely extended grazing land and the presence of many watering points in the river path of the valleys which reduce the concentration and close contact of camels. Moreover, the good practice of herders' timely culling of aborted and non-conceiving females from the herds might have contributed to the situation.

Our result is in accordance with the findings of Abbas and Agab (2002) who reported low seroprevalence (less than 5%) in nomadic or extensively kept camels. The slightly higher seroprevalence in older animals (3.52%) was in line with previous reports of Radostits et al. (2006) which indicated that infection may occur in animals of all age groups but persists commonly in sexually mature animals. Age and sex had no significant effect (P > 0.05) on animal level seroprevalence suggesting existence of susceptibility to brucellosis among male and female camels of different age groups which is in agreement with the previous reports from Ethiopia (Teshome et al., 2003) and Saudi Arabia (Radwan et al., 1992).

The herd (10.27%) and within herd (0 to 7.7%) level seroprevalence reported in the current study is moderately high. The herd level seroprevalence was significantly associated with abortion (P = 0.012) and still birth (P = 0.016). In agreement with our findings Wilson (1998), Tibary et al. (2006), Musa et al. (2008) and Gwida et al. (2011) also reported brucellosis as an important cause of reproductive failure. However, as opposed to our finding, Megersa et al. (2011) reported absence of association between camel brucellosis and abortion at herd level.

The proportions of pregnant (21.8%) and lactating (21.1%) camels in the herd structure reported in current study were very close to the reports of Megersa (2004) and this can be related possibly to camel rearing practices and ecological similarities of the two areas. In this study, the respondents indicated that diseases like trypanosomosis (54%) and anthrax (20%) were causes of abortion in their camels. This was in accordance with Wilson (1998) who suggested trypanosomosis as cause abortion in extensively managed animals.

Conclusion

The present study showed that seroprevalence of camel brucellosis was low. Age, sex, parity, camel rearing experience and herd size had no significant association with *Brucella* seropositivity at animal level. However, at herd level, *Brucella* seropositivity was significantly associated with abortion and still birth. Although seroprevalence of

camel brucellosis is low, the seropositive animals may serve as future foci of infection, pose public health risk, leads to low productivity and market value of camels. Trypanosomosis was among the widespread camel diseases leading to abortion. Further epidemiological studies leading to improvement of health and management of camels and education of pastoralists are imperative to fully exploit the camel resources of the areas.

ACKNOWLEDGEMENTS

REFERENCES

Abbas B, Chabeuf N, Saint-Martin G, Bonnet P, Millaird A, Bashir H, Musa BE (1992). Camel pastoralism in the Butana and Northeastern Sudan: An interdisciplinary study. Nomad. People 31:64-84.

Abbas B, Agab H (2002). A review of camel brucellosis. Prev. Vet. Med. 55:47-56.

Abbas B, Tilley P (1990). Pastoral management for protecting ecological balance in Halaib district, Red Sea province, Sudan. Nomad. People 29:77-86.

Alton GG, Jones LM, Pietz DE (1975). Bacteriological and serological methods. WHO Laboratory Techniques in Brucellosis, 2nd ed. Geneva. pp. 23-124.

Baumann MPO, Zessin KH (1992). Productivity and health of camels (*C. dromedrius*) in Somalia associations with trypanosomiasis and Brucellosis. Trop. Anim. Health Prod. 24:145-156.

CSAE (Central Statistics Authority of Ethiopia) (2004). The 2001/2002 Ethiopian Agricultural Sample Enumeration (EASE). Executive Summary. Addis Ababa, Ethiopia.

Dawood AH (2008). Brucellosis in camels (*Camelus dromedorius*) in the South Province of Jordan. Am. J. Agric. Biol. Sci. 3:623-626.

Demeke S, Kumsa T (1997). Factors to be considered in the formulation of livestock breeding policy. Proceedings of the 5th Conference, Ethiopian Society of Animal Production, Addis Ababa, Ethiopia. pp. 13-27.

Dominech J (1977). Enquête sérologique sur la Brucellose de dromadaire en Ethiopie. Rev. Elev. Méd. Vét. Pays Trop. 30:141-142.

EL-Boshy AH, EL-Khodery S, Osman S (2009). Cytokine response and Clinicopathological findings in *Brucella* infected camels (*Camelus dromedarius*). Vet. Med. 54:25-32.

Gahanem YB, El-khodery SA, Saad AA, Abdelkader AH, Haybe A, Muse A (2009). Seroprevalence of camel brucellosis (*Camelus dromedaries*) in Somaliland. Trop. Anim. Health Prod. 41:1779-1786.

Gwida M, El-Gohary A, Melzer F, Khan I, Rösler U, Neubauer H (2011). Brucellosis in camels. Res. Vet. Sci. (in press).

Higgins AT, Allen WR, Mayhew IG, Snow DH, Wode J (1992). An introduction to the camel in health and disease. Proceedings of the 1st International Camel Conference. R&W Publications, London. pp. 17-19.

Mantur BG, Amarnath SK, Shinde RS (2007). Review of clinical and laboratory features of human brucellosis. Indian J. Med. Microbiol. 25:188-202.

Megersa B (2004). Seroepidemiological study of brucellosis in camels (*Camelus dromedarius*) in Borena lowland pastoral areas, Southern Ethiopia. MSc. thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia.

Megersa B, Molla B, Yigezu L (2005). Seroprevalence of brucellosis in camels (*Camelus dromedaries*) in Borena lowland, Southern Ethiopia. Bull. Anim. Health. Prod. Afr. 53:252-257.

Megersa B, Biffa D, Abunna F, Regassa A, Godfroid J, Skjerve E (2011). Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. Trop. Anim. Health Prod. 43(3):651-656.

Musa MT, Eisa MZ, El Sanousi M, Abdel Wahab EM, Perrett L (2008). Brucellosis in Camels (*Camelus dromedarius*) in Darfur, Western Sudan. J. Comp. Pathol. 138:151-155.

NMAEJB (2006). National metrological Agency of Ethiopia Jijiga branch Annual Report.

OIE (2004). Bovine brucellosis. Manual of Standard for Diagnostic Tests and Vaccines, 5th ed. OIE, Paris. pp. 242-262.

Omer MK, Skjerve E, Holsad G, Woldehiwot Z, Macmillan AP (2000). Prevalence of antibodies to *Brucella* species in cattle, sheep, goats, horses and camels in state of Eritrea. Epidemiol. Infect. 125(2):447-453.

Omer MM, Abdul-Aziz AA, Abusalab MAS, Ahmed MA (2007). Survey of brucellosis among sheep, goats, camels and cattle in Kassala area, Eastern Sudan. J. Anim. Vet. Adv. 6:635-637.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2006). Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th ed. Saunders, London. pp. 963-984.

Radwan AI, Bekairi SI, Prasad PVS (1992). Serological and bacteriological study of brucellosis in camels in Central Saudi Arabia. Rev. Sci. Tech. 11(3):837-844.

Richared D (1980). Dromedary pathology and productions. Provisional Report No. 6. International Science Foundation (IFS), Khartoum, Sudan and Stockholm. Camels 12(18-20):409-430.

Skalsky K, Yahav D, Bishara J, Pitlic S, Leibovici L (2008). Treatment of human brucellosis: Systematic review and meta analysis of randomized controlled trials. BMJ 336:678-679.

Teshome H, Molla B, Tibbo M (2003). A serosurveillance study of camel brucellosis in three camel rearing regions of Ethiopia. Trop. Anim. Health Prod. 35:381-389.

Thrusfield M (2005). Veterinary Epidemiology, 3rd ed. Blackwell Science Ltd, London. pp. 228-242.

Tibary A, Fite C, Anouassi A, Sghiri A (2006). Infectious causes of reproductive loss in camelids. Theriogenology 66:633-647.

Wilson TR (1984). The camel. Longman Group Ltd. London. pp. 118-131.

Wilson TR (1998). Camels. Macmillan Education Ltd., London. P 134.

Yagil R (1985). The Desert Camel: Comparative Physiological Adaptation. Basel Karger, Switzerland. pp. 2-18. (htpp// population mongabay.com/)

Full Length Research Paper

Prevalence, cyst characterization and economic importance of bovine hydatidosis in Mekelle municipality abattoir, Northern Ethiopia

G. Dawit[1,2], A. Adem[4], K. Simenew[1,3]* and Z. Tilahun[1]

[1]College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit, Ethiopia.
[2]College of Agriculture, Aksum University, Aksum, Ethiopia.
[3]School of Agricultural Sciences, Dilla University, Dilla, Ethiopia.
[4]College of Veterinary Medicine, Haramaya University, Ethiopia.

A cross-sectional survey of bovine hydatidosis was carried out on 840 local zebu cattle slaughtered at Mekelle municipal abattoir to determine the prevalence, fertility of hydatid cysts and to assess economic loss. The total prevalence rate was found to be 28.09% at the study period of 8 months from October to May. Observation during the survey period also revealed that the infection rate among different age groups of examined animals were found to be statistically significant (p<0.05), with the highest in old aged cattle (31.98%) followed by adult (21.63%) and young (17.65%). There was statistically significant difference between infection rate and body condition score of the animals with 37.24% lean, 26.27% medium and 21.64% fat body condition. More than 98% of the infected organs were lungs and livers, with higher prevalence in lungs than liver. Out of the total 949 cyst identified, 65.54% were found in lung, 32.88% in liver, 1.01% in heart and 0.53% in kidney. Four hundred and eighty nine of the cysts were small, 160 were medium, 180 were large and 115 were calcified. The fertile, sterile and calcified cysts were found to be 17.44, 45.27 and 37.29%, respectively. Twenty three percent of the fertile cysts were viable and the rest were not. The total annual economic loss was estimated to be 5,200 US Dollar. Furthermore, attempts were made to correlate the origin of the animal and there was no significance between highland and lowland areas.

Key words: Abattoir, economic loss, hydatidosis, prevalence, zebu cattle.

INTRODUCTION

Echinococcosis/hydatidosis is a zoonotic disease that occurs throughout the world and causes considerable economic losses and public health problems in many countries (Daryani et al., 2006). The disease caused by *Echinococcus granulosus*, cystic echinococcosis, is one of the neglected zoonotic diseases recognized. It represents a significant global human disease burden in resource poor pastoral communities (WHO, 2011). This multi host parasite is prevalent all over the world, and annually, the economic loss in livestock due to this para-

site is significant (Lahmar et al., 2004; Tappe et al., 2011). Hydatidosis or larval *Echinococcus* is defined as the cystic stage of *Echinococcus,* a very small tapeworm of dogs and canids. At its intermediate stage, it forms cysts in the internal organs, especially in liver and lungs and some infections can be fatal in humans if the cyst ruptures and causes anaphylactic shock (Acha and Szyfres, 2003; Eckert and Deplazes, 2004; CFSPH, 2011).

E. granulosus and *Echinococcus multilocularis* are the most important members of the genus in respect of their economic loss, public health significance and their geographical distribution. Approximately 60 to 70% of *E. granulosus* cysts occur in the liver and 20 to 25% in the

*Corresponding author. E-mail: drsimenew@yahoo.com.

lungs. The remaining cysts can be found almost anywhere in the body including the bones, kidneys, spleen, muscles, central nervous system (CNS) and behind the eye (CFSPH, 2011). The definitive host is infected by ingestion of offal containing fertile cysts and the intermediate hosts are infected by ingesting contaminated feeds, and water with dog feces contains egg of the parasite (Acha and Szyfres, 2003; Jenkins, 2004). The cycle is completed when an intermediate host or its infected organ is eaten by a suitable carnivore (Thompson and McManus, 2002), and man is usually a dead end intermediate host (Zhang et al., 2003).

Different studies have shown that cystic echinococcosis (*E. granulosus*) represented considerable economic and public health significance in different countries (Azlaf and Dakkak, 2006; Berhe, 2009; Kebede et al., 2009). Present estimates suggest that cystic hydatid disease, caused by *E. granulosus*, results in the loss of 1 to 3 million disability-adjusted life years per annum. The annual cost of treating cases and economic losses to the livestock industry probably amounts to 2 billion US$. *Alveolar echinococcosis* caused by *E. multilocularis,* results in the loss of about 650,000 disability adjusted life years per year as reported by WHO (2011).

In Africa, hydatid disease is reported more commonly in cattle, which are communally owned or raised on free range, and associated more intimately with domestic dogs. Hydatidosis in domestic ruminants inflicts enormous economic damage due to the condemnation of affected organs and lowering of the meat, milk and wool production. In Ethiopia, hydatidosis have been known and documented as early as 1970s. Hydatidosis is the major cause of organ condemnation in most Ethiopian abattoirs and leads huge economic losses (Berhe, 2009; Kebede et al., 2009; Fikire et al., 2012; Terefe et al., 2012).

Certain deeply rooted traditional activities could be commonly described as factors substantiating the spread and high prevalence rates of the disease. These include the wide spread back yard animals slaughter practice, the absence of rigorous meat inspection procedure and the long standing habit of most Ethiopian people to feed their dogs with condemned offal which in effect facilitate the maintenance of the perfect life cycle of *Echinococcus* (Kebede et al., 2009). Despite the large efforts that have been put into the research and control of echinococcosis, it still remains a disease of worldwide significance. In some areas of the world, cystic echinococcosis caused by *E. granulosus* is a re-emerging disease in places where it was at low levels (Torgerson et al., 2002; Torgerson and Budke, 2003; Endrias et al., 2010; CFSPH, 2011).

The fertility of hydatid cysts occurring in various intermediate host species is one of the most important factors in the epidemiology of the disease (Acha and Szyfres, 2003). The fertility of hydatid cysts varies depending on intermediate host species and geographical areas (Saeed et al., 2000; Mcmannus and Smith, 2006), and the status of the problem is not well known especially in developing countries (Torgerson et al., 2002). Despite these, the recent status of hydatidosis in bovine and its economic impact is not well known at different areas of Ethiopia including Mekelle municipal abattoir; though very few attempts were made before 4 years back. Therefore, it is necessary to determine the situation on annual bases. The objectives of this study were therefore to estimate the prevalence and fertility of hydatidosis in bovine slaughtered at Mekelle municipal abattoir and determine the economic impact due to direct and indirect loss during the study period.

MATERIALS AND METHODS

Study area and animals

The study was conducted in Mekelle districts of the Tigray regional state located in the Northern Ethiopia. Mekelle is the largest town of the region and is 783 km North of Addis Ababa. The climate is highland and conducive for animals rearing. The annual average rainfall is 506.47 mm. The annual minimum and maximum temperature was 11.89 to 26.49°C (CSA, 2011).

The study animals comprised indigenous zebu cattle slaughtered at Mekelle municipal abattoir. The slaughtered animals were originated from the Central highlands and from Southern lowlands (Alamata, Raya, Azobo and Mekonni). All cattle presented for slaughter were local breed. The sample size for bovine is calculated according to Thrusfield (2007) by considering 50% expected prevalence and 95° confidence interval with a 5% desired absolute precision. The calculated sample size is 384 and for the higher accuracy, the total numbers of sampling animals were increased to 840. It could have been possible to consider the prevalence in that specific abattoir to calculate the sample size. However, we prefer the 50% to maximize our sample size.

Study design and methodology

A cross sectional study design was followed to study the prevalence hydatidosis from the study animals slaughtered in Mekelle abattoir.

Ante-mortem inspection

Each week, three days visit was made for ante-mortem inspection on individual animals for assessment of animals' origins, age and body conditions. During every visit, each animal were identified based on enumerated marks on its body tagging before slaughter. Animal origin was also recorded as highland (>1500 masl), from central highlands and lowland (<1500 masl), from Southern lowlands (Alamata, Raya, Azobo and Mekonni). The age of the animals was estimated on the basis of the dentitions (Kelly, 1975) and was conventionally classified as young (<2 years), adult (2 to 5 years) and old (>5years). The method described for zebu cattle body condition were three: lean (1 to 3), medium (4 to 6) and fat (7 to 9), and were used based on Nicholson and Butterworth (1986).

Post-mortem examination

Following a through visual inspection, palpation and incision of

Table 1. The distribution and number of organs infected with hydatid cysts from cattle slaughtered at Mekelle municipality abattoir.

Organ	No. infected organs	Frequency (%)
Lung only	127	53.81
Liver only	37	15.68
Lung and liver	63	26.69
Heart	3	1.27
Kidney	2	0.85
Lung and kidney	2	0.85
Lung and heart	1	0.42
Lung, liver and kidney	1	0.42
Total	236	100

suspected organs such as liver, lung, heart and kidney and all the hydatid cyst found in these organs were collected to conduct cyst measurement, cyst count and cyst fertility according to Macpherson (1985). The diameter of the collected hydatid cysts was measured and classified as small (diameter <4 cm), medium (diameter between 4 and 8 cm) and large (diameter >8 cm) (Schantz, 1990; Oostburg et al., 2000).

The collected hydatid cysts were taken to Mekelle regional laboratory. Individual cyst was carefully incised and examined for protoscolices which look like white dots on the germinal epithelium; such cysts were characterized as fertile cysts (Soulsby, 1982). Fertile cysts were subjected for viability test. A drop of the sediment, containing the protoscoleces was placed on microscopic glass slide and covered with coverslip and observed for amoeboid like movement (flame cell activity) with 40X objective. A drop of 0.1% aqueous eosin solution was added to equal volume of protoscolices in hydatid fluid on a microscopic slide with the principle that protoscolices should completely or partially exclude the dye while the dead once take it up (Macpherson, 1985). Furthermore, infertile hydatid cysts were classified as sterile or calcified by their smooth inner lining usually with slight turbid fluid in its content. Typical calcified cysts produce a gritty sound feeling upon incision (Soulsby, 1982; Parija, 2004).

Financial loss assessment

Annual cost of the condemned organs due to bovine hydatidosis was assessed using the following formula set by Ogunirade and Ogunirade (1980). The mean retail market price of condemned organs due to hydatidosis such as liver (35 Ethiopian Birr (ETB)), lung (10 ETB), heart (7 ETB) and kidney (6 ETB) were the parameters considered.

Direct organ condemnation

Annual economic loss due to organ condemnation = $(PI_1 \times T_k \times C_1) + (PI_2 \times T_k \times C_2) + (PI_3 \times T_k \times C_3) + (PI_4 \times T_k \times C_4)$

where: PI_1 = Percent involvement of liver out of the total examined; PI_2 = Percent involvement of lung out of the total examined; PI_3 = Percent involvement of heart out of the total examined; PI_4 = Percent involvement of kidney out of the total examined; C_1 = Average market price of liver; C_2 = Average market price of lung; C_3 = Average market price of heart; C_4 = Average market price of kidney; T_k = Average annual kill of bovines; Carcass weight loss due to hydatidosis.

Five percent estimated carcass weight was lost due to hydatidosis

(Polydorous, 1981), slaughter rates of animals at Mekelle municipal abattoir, average carcass weight (dressing percentage) of Ethiopian zebu cattle breed was 126 kg and the carcass value of beef during the study period was about 32 ETB/kg. The annual carcass weight loss due to hydatidosis was:

ACW = CSR × CL × BC × P
ACW = CSR ×126 × 5% × BC × P

Where: ACW = Annual cost from carcass weight loss; CSR = average slaughtered cattle per annual in the abattoir; CL = carcass weight loss in the individual = (126×5%); BC = average price of 1 kg carcass at Mekelle town; P = prevalence rate of hydatidosis at Mekelle municipal abattoir.

Therefore, the total financial loss due to hydatidosis was the sum of organ condemned (direct) and the cost of carcass weight (indirect) losses.

Data analysis

Data collected from post-mortem, laboratory findings and other factors like age, body condition and origin were entered into MS Excel sheet for storage; Statistical Package for Social Science-15 (SPSS Inc., Chicago, IL, USA) was employed for the analysis. The significant cut point is set to be 0.05. The result is presented in tabulations and narrations.

RESULTS

The present study revealed that the prevalence of bovine hydatidosis at Mekelle municipal abattoir was found to be 28.09%. The distribution and number of organs infected with hydatid cysts in cattle were described and additionally mixed infestation rates were also calculated. The appearance of the cyst in one animal more than one organ also is common where majority of the cysts lodge on the lungs and liver (Table 1).

Observation during the survey period also revealed that the infection rate among different age groups of examined animals were found to be statistically significant (p<0.05) with the highest in old aged cattle (31.98%), followed by adult (21.63%) and young (17.65%). There was statistically significant difference

Table 2. The prevalence and effect of risk of hydatidosis based on different variable categories in cattle slaughtered in Mekelle municipal abattoir.

Variable categories	Number of examined	Number of positive	Prevalence (%)	95% CI	P value
Body condition					
Lean	196	73	37.24	37.24±1.67	
Medium	510	134	26.27	26.27±1.52	0.06
Fat	134	29	21.64	21.64±1.42	
Age					
Young	17	3	17.65	17.65±1.32	
Adult	282	61	21.63	21.63±1.42	0.02
Old	541	173	31.98	31.98±1.61	
Origin					
Highland	559	158	28.26	28.26±1.55	0.86
Lowland	281	78	27.76	27.76±1.55	

between infection rate and body condition score of the animals with 37.24% lean, 26.27% medium and 21.64% fat body condition (Table 2).

Most of the cysts were sterile accounting for 45.27%, and 37.29 were calcified whereas 17.44% were fertile cysts. The cysts were classified as small, medium, large and calcification as described in Figure 1.

The study revealed that in relation to other organs, lungs and liver are the most commonly affected and rejected from local market place, and costing too much loss to the livestock industry of the area. This holds true as in this was reported. The rejection rate of heart, spleen and kidney was however not as significant as that of lungs and liver.

Annual economic loss was determined by considering annual slaughter rate of cattle and prevalence of hydatidosis, and calculated to be 50,060.47 Ethiopian Birr (2,800 USD) due to organ condemnation. A total of about 306 organs were condemned during the study period as shown in Table 3 above. A 5% carcass weight loss due to hydatidosis (Polydorous, 1981) was considered and average total number of slaughtered animals in Mekelle municipality abattoir were 7600 and resulted 42,900.48 Ethiopian Birr (2300 USD) per annum. Total economic loss in the abattoir was 92960.95 Ethiopian Birr (5100 USD) per annum due to both direct and indirect economic loss. This economic loss is increased from the previous study even if the prevalence is almost the same due to increasing cost of organs (offals) and increased number of animals slaughtered in the abattoir. The result of the study shows that the diseases among animals were highly distributed.

DISCUSSION

This study showed similar conclusion which signifies high

prevalence of the disease. From a study conducted in Tigray region reported that echinococcosis/hydatidosis is considerably a prevalent disease in cattle. From 5,194 cattle examined at slaughter houses, 1146 (22.1%) of them were found harboring hydatid cyst (Kebede et al., 2009). The prevalence of cattle hydatidosis study on cattle slaughtered in Mekelle municipal abattoir was 32.1% (Berhe, 2009), Addis Ababa abattoir enterprise was 19.7% (Fikire et al., 2012), Ambo municipal abattoir was 29.69% (Endrias et al., 2010), Dessie municipal abattoir was 13.61% (Melaku et al., 2012) and Kebede et al. (2009a, 2009b) found a prevalence of 34.5 and 16% hydatid cysts in cattle slaughtered in Bahir Dar and Wolaita Sodo abattoir, respectively. The variation in prevalence rate within the same species of animals could be attributed to the differences in seasonal variation, geographical locations and strain differences. Even if monthly studies data report is not included in this research, the prevalence of hydatidosis varies form year to year and from place to place may be ascribed to differences in environmental conditions, hygienic status of slaughter houses, climatic conditions, contamination rate in the intermediate host, dog in each place, slaughtering manner and feeding status of animals, livestock stocking intensity and livestock movement that contribute to the differences in prevalence rates (Njoroge et al., 2002; Tappe et al., 2011). However, the monthly reports interpretation does not signify the actual infestation in that particular month since the disease has chronic nature and makes difficult as to which months of the year the animals acquire the disease.

In the fertility study of the parasite, the rate in the current study is very high as compared to previous reports. In other study, the percentage of fertile cysts: 31.39 (Endrias et al., 2010), 19.3 (Fikire et al., 2012), 10.66 (Berhe, et al., 2010) and 14.95% (Terefe et al., 2012) were documented. Information about prevalence
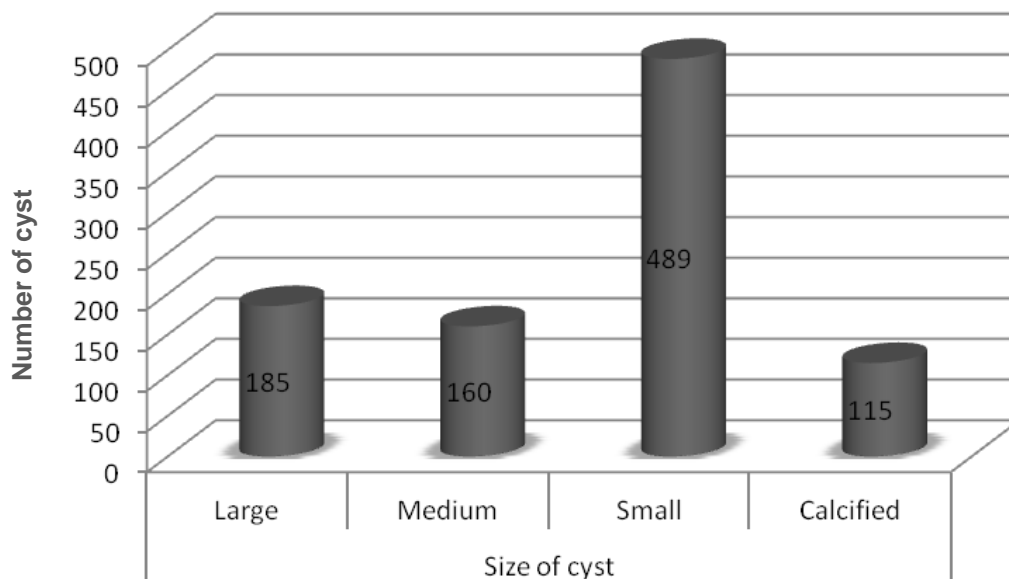
Figure 1. Hydatid cyst size characterization from different organs of cattle slaughtered at Mekelle municipality abattoir.

Table 3. Total number of organs condemned due to bovine hydatidosis in Mekelle municipality abattoir.

Organs condemned	Number of organs condemned	Prevalence (%)
Lung	194	63.82
Liver	101	33.22
Kidney	5	1.64
Heart	4	1.316
Total	304	100

and fertility of hydatid cysts in various organs of cattle are important indicators of potential source of infection to per-petuate the disease to dogs (Endrias et al., 2010). Geno-type of infecting strain affects the fertility rate of the cysts in the intermediate hosts and thereby the infectivity of strain for subsequent hosts (Mwambete et al., 2004). The fertile cyst was found higher in the lung due to soft con-sistency and favors to development, but the per-centage of calcified cyst was found to be higher in the liver than in the lung. This may be associated with the higher reti-culoendothelial cell and abundant connective tissue re-action of the organ (Gemmel and Lawson, 1986). The high proportion of small cysts may be due to immu-nological response of the host, which might preclude exp-ansion of cyst size (Larrieu et al., 2001).

Organ condemnation was commonly seen in this study due to hydatidosis in majority of the cases. Hydatidosis is the major cause of organ condemnation next to *Fasciola* and the first cause of lung condemnation in most Ethiopian abattoirs. In general, the widespread practice of offering dogs with uncooked infected offal, the absence of

well-constructed abattoir and the habit of leaving the dead unburied are important factors that favor the main-tenance and widespread existence of the disease in the study areas (Kebede et al., 2009).

In such areas, bovine hydatidosis in domestic animals can result in significant production losses, including re-duction in live weight gain, yield of milk, fertility rates, the value of hide and skin and in decreased edible offals (Torgerson and Budke, 2003). In addition to losses in-curred in the abattoir, hydatidosis could have economic impact due to invisible losses like impaired productivity; for example, reduced traction power of oxen which re-sults in reduced crop production (Endrias et al., 2010). The economic loss seems lower especially for countries with high prevalence of the parasite. However, it is a huge loss per animal annually in a country like Ethiopia where the daily earning per capita is less than one dollar. It is necessary to carry out epidemiological investi-gations such as possible chain of infections between the final and intermediate hosts and the role of wild animals in the life cycle of the parasite under local conditions are necessary

Parasites in animals can regulate the disease. However, collaboration between veterinarian and public health workers is essential for successful control of hydatidosis (Thompson and Allsop, 1988; WHO, 2011).

CONCLUSION AND RECOMMENDATIONS

Hydatidosis is one of the most highly prevalent parasitic diseases of cattle in Ethiopia and incurring huge eco-nomic loss due to organ condemnation. In view of the present findings and available information, the following recommendations are forwarded. Awareness generating programs should be given for the butchers, abattoir workers, and dog owners to the dangers of hydatidosis to human and animal health. Appropriate control measure should be taken to stop the sale of infected offals for pet animals' consumption. Further studies including geno-typing of the parasite species in that abattoir should be conducted. Government should give attention and building abattoirs with good facilities and control back yard slaughtering.

REFERENCES

Acha PN, Szyfres B (2003). Pan American Health Organization (PAHO): Zoonoses and communicable diseases common to man and animals. Vol. 3. Parasitoses. 3rd ed. Washington DC: PAHO. Scientific and Technical Publication No. 580. Hydatidosis pp 184-199.

Azlaf R, Dakkak A (2006). Epidemiological study of the cystic echinococcosis in Morocco. Vet. Parasitol. 137(1-2):83-93.

Berhe G (2009). Abattoir survey on cattle hydatidosis in Tigray region of Ethiopia. Trop. Anim. Health Prod. 41(7):1347-1352.

Center for Food Security and Public Health (CFSPH) (2011). Echinococcosis. Lowa State of University, College of Veterinary Medicine, Lowa pp 1-14.

Central Statistical Agency (CSA) (2011). The Federal democratic re-public of Ethiopia central statistical agency, agriculture in figure key findings of the 2008/09-2010/11 agricultural sample surveys for all sectors and seasons country summary, FDRECSA Addis Ababa, Ethiopia pp 14-18.

Daryani A, Aiaei R, Arab R, Sharif M (2006). Prevalence of hydatid cyst in slaughtered animals in Northwest Iran. J. Anim. Vet. Adv. 5(4):330-334.

Eckert J, Deplazes P (2004). Biological, epidemiological and clinical aspects of Echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev. 17(1):107-135.

Endrias Z, Yechale T, Assefa M (2010). Bovine hydatidosis in Ambo municipality abattoir. West Shewa, Ethiopia. Ethiop. Vet. J. 14(1):1-14.

Fikire Z, Tolosa T, Nigussie Z, Macias C, Kebede N (2012). Prevalence and characterization of hydatidosis in animals slaughtered at Addis Ababa abattoir, Ethiopia. J. Parasitol. Vector Biol. 4(1):1-6.

Gemmell MA, Lawson JR (1986). Epidemiology and control of hydatid disease. In: Biology of Echinococcus and hydatid disease, Thompson RCA, eds., George Allen and Unwin, London pp 189-216.

Jenkins DJ (2004). Echinococcus in Australia. In: Torgerson PS, Haikenou B eds., Echinococcosis in central Asia problems and solu-tions pp 125-130.

Kebede N, Mekonnen H, Wossene A, Tilahun G (2009b). Hydatidosis of slaughtered cattle in Wolaita Sodo abattoir, Southern Ethiopia. Trop. Anim. Health. Prod. 41(4):629-633.

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 W, Ashenafi H, Zewde G, Fikr L (2009). Echinococcosis/hydatidosis: Its prevalence, economic and public health significance in Tigray region, North Ethiopia. Trop. Anim. Health Prod. 41:865-871.

Kelly WR (1975). Age determination by teeth. In: Veterinary Clinical Diagnosis, 2nd ed., Bailliere Tindall, London pp 12-15.

Lahmar S, Debbeka H, Zhang LH, McManus DP, Souissi A, Chelly S, Torgerson PR (2004). Transmission dynamics of the Echinococcus granulosus sheep-dog strain (G1 genotype) in camels in Tunisia. Vet. Parasitol. 121(1-2):151-156.

Larrieu E, Costa MT, 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): 263-272.

Macpherson CNL (1985). Epidemiology of hydatid disease in Kenya. In a study of the domestic intermediate hosts in masailan. Trans. R. Soc. Trop. Med. Hyg. 79(2):209-217.

Mcmannus DP, Smith JD (2006). Hydatidosis. Changing concepts in epidemiology and speciation parasite today pp 163-168.

Melaku A, Lukas B, Bogale B (2012). Cyst viability, organ distribution and financial losses due to hydatidosis in cattle slaughtered at Dessie municipal abattoir, north eastern Ethiopia. Vet. World 5(4):213-218.

Mwambete KD, Ponce-Gordo F, Cuesta-Bandera C (2004). Genetic identification and host range of the Spanish strains of Echinococcus granulosus. Acta Trop. 91(2):87-93.

Nicholson MJ, Butterworth MH (1986). A guide to condition scoring of zebu cattle. International Livestock Centre for Africa, P.O. Box 5689, Addis Ababa, Ethiopia.

Njoroge EM, Mbithi MF, Gathuma JM, Wachira TM, Gathura TM, Magambo JK and Zeyhle E (2002). A study of cystic echinococcosis in slaughter animals in three selected areas of Northern Turkana, Kenya. Vet. Parasitol. 104(1):85-91.

Ogunrinade AFI, Ogunrinade BI (1980). Economic importance of bovine fasciolosis in Nigeria. Trop. Anim. Health Prod. 12(3):155-160.

Oostburg BFJ, Vrede MA, Bergen AE (2000). The occurrence of poly-cystic echinococcosis in Suriname. Ann. Trop. Med. Parsitol. 94(3):247-252.

Parija SC (2004). Hydatid fluid as a clinical specimen for the etiological diagnosis of a suspected hydatid cyst. J. Parasit. Dis. 28(2):64-68.

Polydrous K (1981). Animal health and economic case study. Echinococcosis with reference to Cyprus. Bull. Off. Int. Epizoot. 93(5):981-992.

Saeed I, Kapel C, Saida LA, Willingham L, Nansen P (2000). Epidemiology of Echinococcus granulosus in Arbil province, Northern Iraq, 1990-1998. J. Helminthol. 74(1):83-88.

Schnatz PM (1990). Parasitic zoonosis in perspective. Int. J. Parasitol. 21(2):161-170.

Soulsby EJL (1982). Helminthes, arthropods and protozoa of domesti-cated animals. 7th ed. Lea and Tebiger, Philadelphia, PA, USA pp 119-127.

Tappe KH, Mousavi SJ, Barazesh A (2011). Prevalence and fertility of hydatid cyst in slaughtered livestock of Urmia city, Northwest Iran. J. Parasitol. Vector Biol. 3(2):29-32.

Terefe D, Kebede K, Beyene D, Wondimu A (2012). Prevalence and fin-ancial loss estimation of hydatidosis of cattle slaughtered at Addis Ababa abattoirs enterprise. J. Vet. Med. Anim. Health 4(3):42-47.

Thompson RCA, Allsop CE (1988). Hydatidosis. Veterinary pers-pectives and annotesed Bibliography CAB. Internal pp 1-16.

Thompson RCA, McManus DP (2002). Towards a taxonomic revision of the genus Echinococcus. Trends Parasitol. 18(10):452-457.

Thrusfield M (2007): Veterinary Epidemiology. 3nd ed., Edinburgh, Black Wellsience Ltd. pp 232-240.

Torgerson P, Budke C (2003). Echinococcosis. An international public health challenge: a review. Res. Vet. Sci. 74(3):191-202.

Torgerson P, Shaikenov B, Kuttybaev O (2002). Cystic echinococcosis in Central Asia: new epidemic in Kazakhstan and Kyrgystan. In Craig P and Pawlowski Z eds., Cestode zoonoses: Echinococcosis and cysticercosis, and emergent and global problem. IOS Press, Amsterdam, Netherlands pp 99-105.

WHO (2011). Report of the WHO Informal Working Group on cystic and alveolar echinococcosis surveillance, prevention and control, with the

participation of the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health 22-23 June 2011. Department of Control of Neglected Tropical Diseases WHO, Geneva, Switzerland pp 1-26.

Zhang W, Li J, McManus PD (2003). Concepts in immunology and diagnosis of hydatid disease. Am. Soc. Microbiol. 16(1):18-36.

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

International Conference on Coelenterate Biology, Eilat, Isreal, 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*