Ethiopian Veterinary Journal

Year: 2019 Volume: 23, No. 2



2019

ISSN:1683-6324 (print) ISSN:2221-5034 (online)

Ethiop. Vet. J. is the Official Scientific Organ of the Ethiopian Veterinary Association

Ethiopian Veterinary Journal (*Ethiop. Vet. J.*)

Objectives and Scope

The Ethiopian Veterinary Journal (*Ethiop. Vet. J.*) is a multidisciplinary peer-reviewed journal intended to promote animal health and production of national and regional/international importance. The journal publishes review articles, original research articles, short communication as well as technical notes in English. Under special circumstances, articles in Amharic may be considered for publication.

Editorial Board Members

Editor-in-Chief:
- ✓ Prof. Kassahun Asmare, School of Veterinary Medicine, Hawassa University, Email: ka7588@yahoo.com

Associate Editors:
- ✓ Prof. Hagos Ashenafi, College of Veterinary Medicine and Agriculture, Addis Ababa University, Email: hagosashenafi@aau.edu.et
- ✓ Prof. Alemayehu Lemma, College of Veterinary Medicine and Agriculture, Addis Ababa University, Email: alemayehu.lemma@aau.edu.et

Assistant Editors:
- ✓ Prof. Tadele Tolosa, School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, Email: tadele.tolosa@ju.edu.et
- ✓ Prof. Asefa Asmare, School Veterinary Medicine, Wolaita Sodo University, Email: asefa62asefa62@gmail.com
- ✓ Dr. Balako Gumi, Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Email: balako.gumi@aau.edu.et
- ✓ Dr. Wudu Temesgen, Department of Veterinary Epidemiology and Public Health, College of Veterinary Medicine and Animal Sciences, University of Gondar, Email: wudu.temesgen@uog.edu.et
- ✓ Dr. Asefa Deresa, Ethiopian Public Health Institute (EPHI), Email: d_asefa2000@yahoo.com
- ✓ Dr. Birhanu Hadush, College of Veterinary Sciences, Mekelle University, Email: birhanu.hadush@mu.edu.et

Editorial Advisory Board
- ✓ Andy Catley, Tufts University, E-mail: Andrew.Catley@tufts.edu
- ✓ Berhanu Admassu, Tufts University, E-mail: berhanu.admassu@tufts.edu
- ✓ Getachew Abebe, Food and Agriculture Organization of the United Nations, Addis Ababa, Ethiopia, E-mail: Getachew.Abebe@fao.org
- ✓ Gijs van't Klooster, Food and Agriculture Organization of the United Nations, E-mail: Gijs.Vantklooster@fao.org
- ✓ J. B. Malone, Louisiana State University, Baton Rouge, Louisiana, USA
- ✓ Kurt Peters, Humboldt University, Berlin, Germany
- ✓ Markos Tibbo, Food and Agriculture Organization of the United Nations, Cairo, Egypt, E-mail: markos.tibbo@fao.org
- ✓ Million Mulugeta, CURE/UCLA School of Medicine, Los Angeles, CA, USA
- ✓ Moges Woldemeskel, University of Tennessee, 2407, River Drive, Knoxville, Tennessee 37919, USA
- ✓ P. Dorchies, Ecole Nationale Veterinaire de Toulouse, Toulouse, France
- ✓ Solomon Hailemariam, African Union, Addis Ababa, Ethiopia
- ✓ Takele Argaw, FDA, Washington D.C., USA
- ✓ Teshome Mebatsion Merial & Sanofi Inc. teshome.mebatsion@merial.com
- ✓ Teshome Yehualashet, Tuskegee University, Alabama, USA
- ✓ Yilma Jobre, Food and Agriculture Organization of the United Nations, Cairo,Egypt, E-mail:yilma.jobre@fao.org

Editorial Manager
- ✓ Dr. Tewodros Tesfaye, Ethiopian Veterinary Association, Email: tewot2000@yahoo.com, Addis Ababa, Ethiopia

Editorial assistant
- ✓ Dr Yitagele Terefe, Haramaya University, College of Veterinary Medicine, Email: yitagele@yahoo.com or myitagele.terefe@ufl.edu, P.O. Box 138, Dire Dawa, Ethiopia

EVA Secretariat Staff
- ✓ Dr. Fasil Awol, fasilawol@gmail.com, P.O. Box 2462, Addis Ababa, Ethiopia
- ✓ Dr. Gewado Ayledo, gagagellebo@gmail.com, P.O. Box 2462, Addis Ababa, Ethiopia
- ✓ Mrs. Sihine Demeke, sihinemariam@gmail.com, P.O. Box 2462, Addis Ababa, Ethiopia
- ✓ Mrs. Meron Solomon, smeron97@gmail.com, P.O. Box 2462, Addis Ababa, Ethiopia

Ethiop. Vet. J., is published by the Ethiopian Veterinary Association (EVA)

Copyright © Ethiopian Veterinary Association (EVA)

Ethiop. Vet. J., 2019, 23 (2)
ISSN: 1683-6324

All articles as well as the editorials published in the Ethiopian Veterinary Journal represent the opinion of the author(s) and do not necessarily reflect the official view of the Ethiopian Veterinary Association, the Editorial Board or the institution within which the author(s) is/are affiliated unless this is clearly stated. Furthermore, the author(s) is/are fully responsible for the contents of the manuscript and for any claim or disclaim therein.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the Association.

Cover drawing by Wossene Abay and Yilma Jobre



Ethiop. Vet. J., is the Official Scientific Organ of the Ethiopian Veterinary Association

Table of Contents

PFGE XbaI® Indistinguishable properties of Salmonella Kastrup and Salmonella Larochelle isolates at beef processing and distribution continuum
Adem Hiko, Getahun E. Agga, Lieselotte Bräutigam, Herlinde Irsigler, Gobena Ameni, Fries Reinhard.....1
https://dx.doi.org/10.4314/evj.v23i2.1

Prevalence of gastrointestinal parasites of dogs and community awareness about zoonotic diseases in Chagni town, northwestern Ethiopia
Nigatu Kebede.....13
https://dx.doi.org/10.4314/evj.v23i2.2

Helminth infection of layer and dual-purpose chickens kept under small scale intensive production system, Ethiopia
Meskerem Adamu, Kasech Melese, Mekonnen Germa, Tewodros Fekadu and Seid Ali.....27
https://dx.doi.org/10.4314/evj.v23i2.3

Bovine brucellosis: Seroprevalence and its potential risk factors in smallholder dairy farms in Hawassa Town, Southern Ethiopia
Ataro Abera, Yosef Deneke, Tadele Tolosa.....41
https://dx.doi.org/10.4314/evj.v23i2.4

Prevalence of bovine trypanosomosis and assessment of knowledge and practices of livestock owners in the control of Trypanosomosis in Assosa District of Benishangul Gumuz Regional State, Ethiopia
Fantahun Miteku, Wagari Tafese and Tsegaw Fentie.....64
https://dx.doi.org/10.4314/evj.v23i2.5

Assessment of retrospective rabies suspected cases registered at two hospitals, community and traditional healers' knowledge, attitude and practices in south Ethiopian pastoralist.
Balako Gumi ,Sisay Girma, Hussein Mohamed, Oudessa Kerro and Hassen Chaka.....77
https://dx.doi.org/10.4314/evj.v23i2.6

Abomasal nematode parasites in goats slaughtered in Mekelle town, northern Ethiopia
Kassaye Berhe and Kassaye Aragw.....90
https://dx.doi.org/10.4314/evj.v23i2.7

Short communication: Important role of Non Tuberculous Mycobacteria in Non-Human primate TB testing in Ethiopia
Rea Tschopp and Liya Wassie.....101
https://dx.doi.org/10.4314/evj.v23i2.8

Guidelines for authors.....107

PFGE *XbaI*® Indistinguishable properties of *Salmonella* Kastrup and *Salmonella* Larochelle isolates at beef processing and distribution continuum

Adem Hiko[1-3], Getahun E. Agga[4], Lieselotte Bräutigam[1], Herlinde Irsigler[1], Gobena Ameni[2], Fries Reinhard[1]*

[1]Institute of Meat Hygiene and Technology, Panel Veterinary Public Health and FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Brümmerstraße 10, 14195 Berlin, Germany.

[2]Akililu Lemma Institute of Pathobiology, 1176 Addis Ababa University, Ethiopia.

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

[4]United States Department of Agriculture, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166.

Abstract

Salmonellosis is a major cause of foodborne illnesses in humans throughout the world. Pulsed field gel electrophoresis (PFGE) is a commonly used tool for epidemiological survey and source tracking of *Salmonella* strains isolated from various sources. A total of 237 samples from various sources were collected at abattoir and butcheries in Ethiopia from December 2011 to April 2012. Isolates of *Salmonella* Kastrup (n = 3) and *Salmonella* Larochelle (n = 11) were characterized for antimicrobial resistance using disc diffusion and for genotypic profiles using PFGE *XbaI*®. *Salmonella* Kastrup and *S.* Larochelle were isolated from 1.3% and 4.6% of total samples (n = 237) examined. Except for water samples (n = 12) and truck swabs (n=11), all other sample sources were positive for one or more of *S.* Kastrup and *S.* Larochelle. *Salmonella* Kastrup was isolated in Ethiopia for the first time. *Salmonella* isolates from both Kastrup and Larochelle serotypes showed similar antimicrobial resistance profiles. Two pulsotypes consisting of the same three *S.* Kastrup and 10 *S.* Larochelle cluster and second cluster consisting of one *S.* Larochelle were identified at 1.5% and 1% band positions tolerance by unweighted-pair group method (UPGM) and showed ≥91% similarity. *S.* Kastrup and *S.* Larochelle isolates showed similar antimicrobial resistance profiles with indistinguishable PFGE *XbaI*® patterns indicating possible clonal spread of the isolates along beef processing and distribution continuum.

Key words: Salmonella; PFGE; Antimicrobial resistance; Beef; Ethiopia

Introduction

Widespread distribution of *Salmonella* in the environment, their increase in prevalence in the global food chain, and their virulence and adaptability properties cause easy transmission resulting in enormous medical, public health and economic impact worldwide (Molbak *et al.,* 2006). Serology based on surface antigens (Grimont and Weill, 2007), phage typing based on bacteriophage host profile (Jay *et al.,* 2005), antimicrobial susceptibility; and biotyping, which uses biochemical tests to reflect metabolic activities of *Salmonella* strain (WHO, 2010), are used for phenotypic characterization. Pulsed field gel electrophoresis (PFGE) and whole genome sequencing are the most commonly applied genotyping techniques for epidemiological investigations of food borne gram-negative pathogens including *Salmonella* (Foley *et al.*, 2009; Miller, 2013; PulseNet, 2013). PFGE is based on the whole chromosomal DNA digestion with the use of one or more specific restriction endonuclease enzyme at selected genomic restriction sites. The restriction digestion results in 8- 25 high molecular weight DNA fragments of 10-800 killobase (kb) in size (Wiedmann, 2002; Jay *et al.*, 2005). Some of the restriction enzymes used for *Salmonella* PFGE are *XbaI, BlnI; SpeI*, *SfiI*, *PacI* and *NotI*. Zheng et al. (2007) showed the differences in discriminatory power of each of these enzymes independently and/or in different combinations. Resulting DNA fragments are then separated by PFGE in agarose gels. The fingerprints derived from the process are analyzed by BioNumerics® software (BioNumerics, 2011) programmes to determine clonal diversity and relationship between the isolates (Olive and Bean, 1999).

In Ethiopia, different *Salmonella* serovars were isolated from animals and humans (Alemayehu et al., 2003; Tibaijuka et al.; 2003; Ejeta et al., 2004; Molla et al., 2004; Aragaw et al., 2007; Zewdu and Cornelius, 2009; Sibhat et al., 2011; Beyene et al., 2011; Hiko et al., 2016; Kiflu et al., 2017; Hiko et al., 2018). However, PFGE analysis of the isolates was not conducted; thus establishing genetic relationships for epidemiological purposes is impossible. Using antigens, *S.* Kastrup (6, 7:e,n,z_{15}:1,6) and *S.* Larochelle (6, 7:e, h:1,2) shows closes while serotyping, (Grimont and Weill, 2007). But, the genotypic *XbaI* PFGE and phynotypic drug resistance profiles of *Salmonella* Kastrup and *Salmonella* Larochelle were not yet assessed in Ethiopia. The objective of the current study was to characterize *Salmonella* Kastrup and *Salmonella* Larochelle isolates obtained from various samples collected from beef processing and distri-

bution continuum in Ethiopia, for their genotypic profiles and antimicrobial resistance profile.

Materials and Method

Ethical Consideration

Samples were also collected from personnel's hands. Taking into consideration that the sampling was less invasive and also the samples were from adults working in abattoirs, individuals involved on the sample were informed regarding the details the research. Hence, informed consent was obtained from the study participants.

Description of beef processing and distribution continuum

The *Salmonella* strains we characterized in the current report were isolated from different sample types as part of a study conducted to investigate *Salmonella* at food processing plants and distribution. Addis Ababa Abattoir Enterprise (AAAE) and butchery which distribute raw beef in Addis Ababa city as well as selected beef processing plant and supermarkets in Addis Ababa city which distribute the processed beef (Mortadella) were investigated for sample collection. Ethical clearance for the study was obtained Ethiopian Engineering Capacity Building in collaboration with Health sector.

Sources of the Salmonella isolates

Swab samples from personnel hands, aprons, knives, hooks, rooms, refrigerators and beef transporting trucks were collected with sterile gauze moistened with normal saline. About 10 ml of tap-water samples were collected from the pipe line. Cattle feces were collected from the rectum; and mesenteric lymph nodes were collected after evisceration. Beef cut samples were collected at abattoir after meat inspection.Similarly, following the unique carcass identification number, raw beef of the respective cattle was sampled at retail butchers' shops (public meat supply station) in Ethiopia (Table 1). Samples were collected from December 2011 to April 2012. Bacterial isolation was conducted at Microbiology Laboratory, Akililu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia following standard protocols (Grimont and Weill, 2007).

Pre-enrichment was done using one portion of sample by volume or gram which was homogenized in 10 portions of buffered peptone water (BPW) (Merck, Germany) at 1:10 proportion. From the pre-enriched samples, 0.1 ml and 1 ml was transferred to 10 ml of Rappaport-Vassiliadis (RV) medium (Oxoid Hampshire, England) and 10 ml of Muller Kaufmann tetrathionate with novobiocin (MKTTn) (Merck) broths respectively for selective enrichment. RV and MKTTn broth cultures were then incubated at 43ºC and 37ºC respectively for 18-24 hrs. A loopful was plated on Brilliant phenol lactose sucrose agar (BPLS) (Merck) and Xylose lactose Tergitol™ 4 (XLT4) (Merck) in parallel and incubated at 37ºC for 24 hrs and 48 hrs, respectively. Presumptive colonies based on their characteristic morphological appearances on the selective agar plates were sub-cultured onto standard-I nutrient agar (Merck) and biochemically using lysine test, TSI test, catalase test, indole production test and citrate utilization were confirmed for serotyping.

Serotyping

The isolates were stereotyped at Microbiology Laboratory, Institute of Meat Hygiene and Technology, Panel Veterinary Public Health, FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Germany. Serotyping was done using *Salmonella* antisera (Sifin, Berlin, Germany) with O-antigen and H-antigen agglutination test (Grimont and Weill, 2007).

Antimicrobial susceptibility testing

All of the isolates were tested for their phenotypic antimicrobial resistance by agar disc diffusion method with antimicrobial impregnated discs (Oxoid, Hampshire, England). The drugs were selected based on its used in health sectors in Ethiopia and availability on the market. The test was done against polymyxin-B (POL-B; 300U), trimethoprim-sulfamethoxazole (STX; 1.25/23.75 µg), chloramphenicol (CHL; 50µg), gentamycin (GEN; 10µg), trimethoprim (TRM; 5µg), neomycin (NEO; 10µg) and oxytetracycline (OxyTET; 30µg). Antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid) according to Bauer Kirby agar disc diffusion (Bauer *et al*., 1966) following Clinical Laboratory Standards Institute's protocol (CLSI, 2007). The isolates were sub-cultured onto standard-I nutrient agar (Merck) and incubated at 37ºC for 24 hrs. They were then inoculated into 3 ml of brain heart infusion broth (BHI) (Merck) and again incubated for 1 hr at 37ºC. The inoculum density was standardized to 0.5 McFarland standards; from which 0.1 ml was spread onto Mueller-Hinton agar (Oxoid). After the plates were allowed to absorb the moisture;

antimicrobial impregnated discs were applied; and the plates were incubated at 35 ± 2°C for 16-18 hr. Based on the diameter of zone inhibition for *Enterobacteriaceae*, results were recorded as susceptible, intermediate or resistant (CLSI, 2007).

Pulsed filed gel electrophoresis (PFGE)

The PFGE was conducted following PulseNet protocol (PulseNet, 2013) at Molecular Biology Laboratory, Institute of Meat Hygiene and Technology, Panel Veterinary Public Health, FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Germany. Agarose-embedded whole genomic DNA of the isolates was digested with *XbaI*® enzymatic restriction using 60U *XbaI*® (Roche Diagnostics GmbH, Germany). DNA fragments were separated by PFGE in agarose containing gels. *S.* Braenderup STSAL82 (Merck, Germany) was used as a reference strain. A 50-1000kb Pulse marker™ (Sigma-Aldrich Co, USA), test strains and reference strain were loaded into 1.2% Pulsed Field Certified Agarose® gel. The gel running condition was set according to Pulse Net (2013) and followed by stained with 1mg/l ethidium bromide solution. Using Digital Imaging and Analysis System II (DIAS-II), the gel image was taken as ".jpg" processed into ".tif" file. The PFGE files were transferred into computer and processed using BioNumerics® software (Applied Maths BVBA, Kortrijk, Belgium).

Data analysis

Results of isolation and antimicrobial susceptibility testing were expressed as percentages. Isolates were compared using genomic cluster analysis method in BioNumerics®. The banding patterns were analyzed by Bionumerics software to determine clonal diversity and relation between the isolates. The cluster analysis was based on a variety of algorithms that have the common feature of hierarchical relatedness between and among the isolates using dendrogram or tree. During analysis, an optimization of 1.0 with three different band position tolerances of 1.5%, 1% and 0.8% by the unweighted-pair group method (UPGM) were used in BioNumerics® software program to assess genomic properties of these isolates.

Results

Distribution of isolates in samples

Out of 237 samples tested, 3 (1.27%) *S.* Kastrup and 11 (4.64%) *S.* Larochelle isolates were isolated (Table 1). *S.* Kastrup was isolated from Ethiopia for the first time. The three *S.* Kastrup isolates were isolated one each from personnel hand swab, animal feces and mesenteric lymph nodes. *S.* Larochelle was isolated from swab samples taken from aprons (1), knife (1), hook (1), refrigerator (1), room floor (2); raw beef (3) and cattle feces (2).

Table 1: Distribution of S. Kastrup and S. Larochelle isolates from various samples obtained from cattle slaughter plant and retail shops

Sample category	Sample source	Sample type	No. samples tested	No. Isolates	Serotypes detected (no.)
Abattoir environment	Personnel's hands	Swab	13	1	S. Kastrup (1)
	Aprons	Swab	14	1	S. Larochelle (1)
	Knives	Swab	13	1	S. Larochelle (1)
	Water	Water sample	12	0	-
	Hooks	Swab	11	1	S. Larochelle (1)
	Rooms	Surface swab	17	2	S. Larochelle (2)
	Refrigerators	Swab	10	1	S. Larochelle (1)
	Trucks	Swab	11	0	-
	Sub total		101	7	S. Kastrup (1); S. Larochelle (6)
Cattle at abattoir	Feces	Fecal samples	34	3	S. Kastrup (1); S. Larochelle (2)
	Mesenteric lymph nodes	Lymph nodes	34	1	S. Kastrup (1)
	Beef	Carcass samples	34	1	S. Larochelle (1)
	Sub total		102	5	S. Kastrup (2); S. Larochelle (3)
Retail shop	Beef at butcheries	Beef samples	34	2	S. Larochelle (2)
	Grand total (no; %)		237	14	S. Kastrup (3; 1.27); S. Larochelle (11; 4.64)

Antimicrobial resistance

Of the 14 isolates, 7.1%, 35.7% and 21.4% of them were resistant to gentamicin, neomycin and oxytetracycline respectively. As shown in Table 2, only two of the three (66.7%) *S.* Kastrup isolates were resistant to neomycin alone and one (9.1%) of *S.* Larochelle isolates was resistant to gentamycin. Three (27.3%) of *S.* Larochelle isolates each were resistant to neomycin and oxytetracycline. Of these three, one isolate was resistant to both drugs. No multiple drug resistance was observed.

Table 2: Antimicrobial susceptibilities of *S.* Kastrup and *S.* Larochelle isolates obtained from various samples at beef processing plant and retail shops

Serotypes	a Resistance category	b Antimicrobial resistance profile, n (%)						
		POL-B	GEN	CHL	TRM	SXT	NEO	OxyTET
S. Kastrup (n = 3)	S	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	1 (33.3)	3 (100)
	I	0	0	0	0	0	0	0
	R	0	0	0	0	0	2 (66.7)	0
S. Larochelle (n = 11)c	S	11 (100)	10 (90.9)	11 (100)	11 (100)	11 (100)	3 (27.3)	8 (72.7)
	I	0	0	0	0	0	5 (45.4)	0
	R	0	1 (9.1)	0	0	0	3 (27.3)	3 (27.3)
Total (n = 14)	S	14 (100)	13 (92.9)	14 (100)	14 (100)	14 (100)	4 (28.6)	11 (78.6)
	I	0	0	0	0	0	5 (35.7)	0
	R	0	1 (7.1)	0	0	0	5 (35.7)	3 (21.4)

[a] S = susceptible; I = intermediate; R = resistant

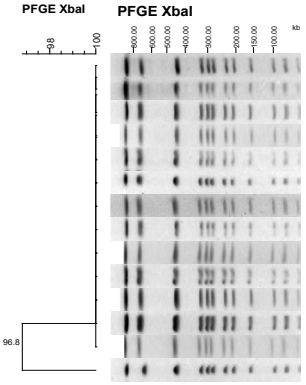
[b] POL-B = polymyxin-B; GEN = gentamicin; CHL= chloramphenicol; TRM =trimethoprim; SXT = trimethoprim-sulfamethoxazole; NEO= neomycin; OxyTET = oxytetracycline

[c] one isolate was resistant to neomycin and oxytetracycline

Pulsed field gel electrophoresis finding

Two pulsotypes with 96.8% similarities at 1.5% band position tolerance (Fig. 1) and with 91.7% similarity at 1% band position tolerance (Fig. 2), both consisting of the same thirteen isolates (three *S.* Kastrup and 10 *S.* Larochelle) of 100% similarity and one *S.* Larochelle were obtained. Using 0.8% band posi-

tion tolerance, four pulsotypes consisting 2, 1 and 1 isolates of S. Larochelle, and 100% similar 10 isolates (3 *S.* Kastrup and 7 *S.* Larochelle) were observed (Fig. 3).



Key	Serovars	Sample	Source	Batch	Month	Domain	Code	pulsoty
533.	S. Kastrup	Hand Sw.	Abattoir	4th	January	Environment	4	SKLX1
535.	S. Kastrup	Fecal	Abattoir	11th	February	Animal	24	SKLX1
537.	S. Kastrup	MLN	Abattoir	17th	April	Animal	29	SKLX1
533.	S. Larochelle	Apron	Abattoir	4th	January	Environment	5	SKLX1
535.	S. Larochelle	Knife	Abattoir	11th	February	Environment	10	SKLX1
536.	S. Larochelle	Hook	Abattoir	17th	April	Environment	-	SKLX1
536.	S. Larochelle	Room	Abattoir	17th	April	Environment	-	SLKX1
536.	S. Larochelle	Meat	Abattoir	17th	April	Animal	29	SKLX1
536.	S. Larochelle	Fecal	Abattoir	17th	April	Animal	29	SKLX1
537.	S. Larochelle	Refrigera.	Abattoir	18th	April	Environment	-	SKLX1
537.	S. Larochelle	Room	Abattoir	18th	April	Environment	-	SKLX1
537.	S. Larochelle	Fecal	Abattoir	18th	April	Animal	31	SKLX1
538.	S. Larochelle	Meat	Butchery	18th	April	Animal	32	SKLX1
538.	S. Larochelle	Meat	Butchery	18th	April	Animal	33	SKLX2

Fig. 1: *Salmonella* Kastrup and *Salmonella* Larochelle using PFGE *XbaI*® endonuclease at 1.5% band position tolerance by the UPGM. Hand Sw = Hand swab; Refrigera = Refrigerator swabs; MLN = mesenteric lymph node; SKLX = *Salmonella*-Kastrup-Larochelle-*XbaI*® palsotype; numbers following SKLX are grouping of palsotype(s).

Discussion

Except for water and beef transporting truck swab samples, all sample sources were positive for one or more *Salmonella* Kastrup and S. Larochelle isolates. In current study, *S.* Kastrup was detected from 1.27% of samples collected from cattle feces, swabs from personnel's hands and mesenteric lymph nodes. This is the first report of *S.* Kastrup from Ethiopia. Menghistu et al. (2011) reported overall prevalence of 2.7% *Salmonella* including *S.* Kastrup from poultry in India, showing epidemiological diversity of this serotype in food animals. The predominant occurrences of S. Larochelle serotype (4.64%) in the food chain including in the production environment, cattle feces and raw beef at different stages of the abattoir line poses risk to the public. Beyene (2008) reported one isolate of *S.* Larochelle from hospital cases in Ethiopia.

The 7.1% gentamicin resistance observed in the current study is similar to 3.6% reported by Reda et al. (2011), but lower than the 74.3% reported by Beyen et al. (2011) in *Salmonella* isolated from human cases in Ethiopia. The low

gentamicin resistance observed here could be due to infrequent use of this drug in food animal in Ethiopia. The 35.7% neomycin resistance we observed was higher than the 6.3% report of Oliveira et al. (2006) in *S.* Enteritidis isolated from foods involved in foodborne outbreaks in Brazil. The 21.4% oxytetracycline resistance observed in the present study was lower than 41.2% (Molla *et al.,* 2003), 94.5% (Asrat, 2008), 71.4% (Reda *et al.,* 2011), 39.8% (Beyene *et al*., 2011), 31.8% (Aragaw *et al.,* 2007) reported from *Salmonella* isolates from humans and animals in Ethiopia. These studies show the widespread occurrence of tetracycline resistance in *Salmonella* isolates from Ethiopia. The Drug Administration and Control Authority of Ethiopia report (DACA, 2009) shows that tetracycline is a commonly used antibiotic in Ethiopia. *S.* Kastrup isolates showed resistance only to neomycin as opposed to *S.* Larochelle isolates which showed resistance to gentamicin and oxytetracycline in addition to neomycin indicating some degree of phenotypic difference.

Although *S.* Kastrup (6,7:e,n,z_{15}:1,6) and *S.* Larochelle (6,7:e, h:1,2) (Grimont and Weill, 2007) are antigenically differentiated by serotyping, our results indicate that the two serotypes demonstrate genetically indistinguishable PFGE *XbaI*® profiles. Using PFGE *XbaI*® as epidemiological tool for tracing possible sources of food borne infections, our observation of 100% genetic similarity among *S.* Kastrup isolates from personnel's hands, mesenteric lymph nodes and cattle feces shows their possible sources for carcass contamination at the studied abattoir. Similarly, the 100% similarity of *S.* Larochelle isolated at abattoir line shows the possible cross-contamination and cross-transmission to the level of meat supply (butchers'). On the other hand, the difference of pulsotype 2 from others and observing from the butchers' (meat supply station) might indicate contamination of carcass during handling and supply.

In conclusion, the finding of indistinguishable PFGE *XbaI*® patterns of *S.* Kastrup and *S.* Larochelle from various samples collected along beef production, processing and retail shops indicates the possibility of transfer of these serotypes along the processing and handling. The prevalence of antimicrobial resistance of these *Salmonella* serotypes was low with similar susceptibility patterns to the drugs tested. *Salmonella* Kastrup was isolated from Ethiopia for the first time with indistinguishable PFGE *XbaI*® profiles from *S.* Larochelle.

Acknowledgments

We would like to thank Dr. Nina Langkabel for transporting *Salmonella* isolates from Addis Ababa to Institute of Meat Hygiene and Technology, Panel Veterinary Public Health, FAO Reference Center for Veterinary Public Health, Freie Universität Berlin, Germany. Cooperation of Dr. Kebebe Erberto during sample collection from Addis Ababa Abattoir Enterprise was highly appreciated.

References

Alemayehu, D., Molla, B., Muckle, A. 2003. Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia. *Trop. Anim. Hlth. Prod*., 35, 309-319.

Aragaw, K., Molla, B., Muckle, A., Cole, L., Wilkie, E., Poppe, C., Kleer, J., Hildebrandt, G. 2007. The characterization of *Salmonella* serovars isolated from apparently healthy slaugh-tered pigs at Addis Ababa abattoir, Ethiopia. *Prev. Vet. Med.,* 82, 252-261.

Asrat, D. 2008. *Shigella* and *Salmonella* serogroups and their antibiotic susceptibility patterns in Ethiopia. *East Mediter. Hlth. J.,* 14(4), 760-767.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. 1996. Antibiotic susceptibility testing by standard single disc method. *Am. J. Clin. Patholo.,* 45(4), 493-496.

Beyene, G. 2008. Phenotypic and molecular characterizations of *Salmonella* species in Ethiopia. A PhD Thesis presented to the School of graduate studies of Addis Ababa University, Addis Ababa, Ethiopia.

Beyene, G., Nair, S., Asrat. D., Mengistu. Y., Engers. H., Wain, J. 2011. Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia. *J. Infect Dev Ctries.,* 5(1), 23-33.

BioNumeric®, 2011. Applied Maths BVBA, Kortrijk, Belgium. http://www.applied-maths.com/bionumerics).

CLSI, 2007. Clinical and Laboratory Standards Institute (CLSI). "Performance standard for antimicrobial disk and dilution susceptibility tests for Bacterial isolation from animal".

DACA, 2009. Antimicrobial use, resistance and containment baseline survey syntheses of findings. Drug Administration and Control Authority, 2009, Addis Ababa, Ethiopia.

Ejeta, G., Molla, B., Alemayehu, D., Muckle, A. 2004. *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Revue. Méd. Vét*., 155, 547-551.

Foley, S.L., Lynne, A.M., Rajesh, N. 2009. Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. Review. *Infect. Genet. Evol.*, 9(4), 430-440.

Grimont, P.A.D., Weill, F. 2007. Antigenic formulae of the *Salmonella* serovars 2007. 9th Ed.. WHO Collaborating Centre for Reference and Research on *Salmonella.* Pp: 1-167.

Hiko, A., Irsigler, H., Bräutigam, L., Ameni, G., Fries, R., Maximilian, B. 2018. Antimicrobial resistance and genotypic profiles of *Salmonella* Saintpaul isolated along beef processing and distribution continuum. *Heliyon*, 4 (12), e01025. doi:10.1016/j.heliyon.2018.e01025

Hiko, A., Irsigler, H., Ameni, G., Zessin, K., Fries, R.. *(2016). Salmonella* serovars along two beef chains in Ethiopia. *The Journal of Infect. Dev. Ctries.,* 10 (11), 1168-1176. doi:10.3855/jidc.6354.

Jay, M.J., Loessner, J.M., Golden, A.D. 2005. Modern food microbiology. 7th Ed. Food Science Text Series. Springer Science, New York, USA.

Kiflu, B., Alemayehu, H., Abdurahaman, M., Negash, Y.,Eguale, T., 2017. *Salmonella* serotypes and their antimicrobial susceptibility in apparently healthy dogs in Addis Ababa, Ethiopia. *BMC Vet. Res.*, 13(1)*,* 134. doi:10.1186/s12917-017-1055-y.

Menghistu, H.T., Rathore, R., Dhama, K., Agarwal, R.K. 2011. Isolation, Identification and Polymerase Chain Reaction (PCR) Detection of *Salmonella* Species from Field Materials of Poultry Origin. *Intl. J. Microbiol. Res.,* 2(2), 135-142.

Miller, M.J 2013. Whole-Genome Mapping: a New Paradigm in Strain-Typing Technology. *J. Clin. Microbiol.,* 51(4), 1066-1070.

Molbak, K., Olsen, J.E., Wegener, H. C. 2006. *Salmonella* infections. In: Foodborne Infections. Foodborne Infections and Intoxications, 3rd ed., 57-114.

Molla, B., Mesfin, A., Alemayehu, D. 2003. Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. *Eth. J. Health Dev.,* 17, 131-149.

Molla, B., Mohammed, A., Salah, W. 2004. *Salmonella* prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedarius*) in Eastern Ethiopia. *Trop. Anim. Health. Prod*., 36, 451-458.

Molla, B., Salah, W., Alemayehu, D., Mohammed, A. 2004. Antimicrobial resistance pattern of Salmonella serotypes isolated from apparently healthy slaughtered camels

(Camelus dromedarius) in eastern Ethiopia. Berl. Munch. Tierarztl. Wochenschr., 117, 39-45.

Olive, D.M., Bean, P. 1999. Principles and Applications of Methods for DNA-Based Typing of Microbial Organisms. J. Clin. Microbiol., 37, 1661-1669.

Oliveira, F.A, Brandelli, A., Tondo, E.C. 2006. Antimicrobial resistance in Salmonella Enteritidis from foods involved in human salmonellosis outbreaks in southern Brazil. New Microbiol., 29(1), 49-54.

PulseNet, 2013. Standard Operating Procedure for PulseNet PFGE of Escherichia coli O157:H7, Escherichia coli non-O157 (STEC), Salmonella serotypes, Shigella sonnei and Shigella flexneri PNL05 Last Updated April 2013.

Reda, A.Y., Seyoum, B., Yimam, J., Andualem, G., Fiseha, S., Vandeweerd, J.-M. 2011. Antibiotic susceptibility patterns of Salmonella and Shigella isolates in Harar, Eastern Ethiopia. J. Infect. Dis. Immun., 3(8), 134-139.

Sibhat, B., Zewde, B.M., Zerihun, A., Muckle, A., Cole, L., Boerlin, P., Wilkie, E., Perets, A., Mistry, K., Gebreyes, W. A. 2011. Salmonella serovars and antimicrobial resistance profiles in beef cattle, slaughterhouse personnel and slaughterhouse environment in Ethiopia**.** Zoonoses Pub. Hlth., 58(2), 1863-2378.

Tibaijuka, B., Molla, B., Hildebrandt, G., Kleer. J. 2003. Occurrence of Salmonella in retail raw chicken products in Ethiopia. Berl Munch Tierarztl Wochenschr., 116, 55-58.

WHO, 2010. A WHO network building capacity to detect, control and prevent foodborne and other enteric infections from farm to table" Laboratory Protocol: "Biochemical Identification of Salmonella and Shigella Using an Abbreviated Panel of Tests" Enteric Diseases Laboratory Branch Centers for Disease Control and Prevention Atlanta, GA; USA. Pp: 1-45

Wiedmann, M. 2002. Subtyping of Bacterial Foodborne Pathogens. Nutr. Rev., 60 (7 Pt.1), 201-208.

Zewdu, E., Cornelius, P. 2009. Antimicrobial resistance pattern of Salmonella serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. Trop. Anim. Helth, Prod., 41(2), 241-249.

Zheng, J., Keys, C.E., Zhao, S., Meng., J., Brown, E.W. 2007. Enhanced Subtyping Scheme for Salmonella Enteritidis. Emerg. Infect. Dis., 13(12), 1932-1935.

Kebede, Ethiop. Vet. J., 2019, 23 (2), 13-26
DOI https://dx.doi.org/10.4314/evj.v23i2.2 *Ethiopian Veterinary Journal*

Prevalence of gastrointestinal parasites of dogs and community awareness about zoonotic diseases in Chagni town, northwestern Ethiopia

Nigatu Kebede[1*]

[1]*Akillu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia*

**Address for the author: E-mail: nigatukebede@yahoo.com, nigatu.kebede@aau.edu.et*

Tel.: +251112763091, Fax: +251112755296, P. O. Box: 150480

Abstract

This cross-sectional study was conducted to estimate the prevalence of gastrointestinal helminth species found in stray dogs and to assess the awareness of the community about dog parasitic zoonoses in Chagni town, northwest Ethiopia. Standard fecal and postmortem examination and questionnaire survey methods were used to collect data. A total of 202 fecal samples were collected and examined using the Kato-Katz methodology to indicate intestinal helminthes in dogs of the study area. Postmortem examination was done on 9 stray dogs to detect the presence of adult worms. Necropsy of the 9 stray dogs revealed that all of them (100%) were positive for at least one intestinal helminth parasite. The following cestodes were identified: *Echinococcus granulosus*, *Taenia pisiformis*, *T. hydatigena*, *T. ovis*, *Dipylidium caninum* and Mesocestoides. Other intestinal worms in dogs were *Toxocaracanis*, *Tirchurisvulpis* and *Ancylostoma caninum*. Most helminths were recovered from the large intestine. Of the interviewed community members, 68.3% reported that tapeworms locally called "*kosso*" were zoonotic agents, followed by roundworms, locally called "*wosefat*". Majority of the respondents reported they feed their own dogs raw condemned offal's and human. The findings showed that there is significant infestation of intestinal helminthes among the dogs of the study area coupled with the high levels of illiteracy among community members about canine parasites and transmission. This shows that immediate action needs to be taken to decrease infection rates in dogs and to raise education levels of the community about zoonotic diseases.

Keywords: Chagni; Dog; Helminthes; Intestinal parasites; Prevalence; Zoonoses

Introduction

Dogs are the most successful canids, adapted to human habitation worldwide (Tannen, 2004), live in close association with humans, providing them with companionship and security, among others (Paul *et al*., 2010). Although dogs bring many advantages to human life, including being guides for blind people, therapeutic agents, security guards, and hunters, they are associated with many potentially zoonotic pathogens including parasites (Chomel, 2014). Gastrointestinal parasites are among the main enteropathogens and the major cause of mortality in dogs. Canine zoonotic helminths pose a public health risk through various ways (Habluetzel*et al*., 2003; Rubel and Wisnivesky, 2005; Dubná*et al*., 2007). Canine and human infection with zoonotic helminths can occur through ingestion of the infective eggs and ingestion or skin penetration of the infective larvae, contact with animal hair (Habluetzel*et al*., 2003; Dubná*et al*., 2007), food and water contaminated with dog excreta or secretions, and/or consumption of dog meat (Cui and Wang, 2001).

Previous studies indicated that canine zoonotic parasites include *Toxocara canis*, *Diphyllobothrium latum, Ancylostoma* spp*., Uncinaria stenocephala* (Joffe *et al*., 2011), and *Echinococcus granulosus* (Abdi *et al.,* 2013). Human infection with *Toxocara* spp. is typically asymptomatic, however, some individuals developed visceral *larva migrans* and ocular toxocariasis (MacPherson *et al.,* 2000; Ahn *et al*., 2014). *Ancylostoma* spp. are agents of cutaneous *larva migrans* (MacPherson *et al*., 2000) and *Ancylostoma caninum* has also been associated with eosinophilic enteritis in humans (Croese *et al*., 1994; Walker *et al*., 1995).

Zoonotic canine gastrointestinal parasites were reported worldwide, 4.4% in Saskatchewan(Gaunt and Carr, 2011), 7.4% in Kerman city, Iran (Mirazaei and Fooladi, 2012),43.3% in Ibadan, Nigeria (Ayinmode *et al*., 2016),52.6% in Ashanti, Ghana (Amissah-Reynolds *et al*., 2016), 62.6% in Accra, Ghana (Johnson *et al*., 2015), 68.4%in Nigeria (Ugbomoiko *et al*., 2008), 75.26 and 84.78% in Bahir Dar, Ethiopia (Abere *et al*., 2013)and 86.8% in Hawassa, Ethiopia(Paulos *et al.,* 2012) were reported. The variation in prevalence reported could be due to differences in status of dog sampled, geographical location, and the diagnostic techniques used (Robertson *et al*., 2000; Oliveira-Sequeira *et al*., 2002). High prevalence and heavy infections with gastrointestinal parasites are more commonly reported in dogs in developing countries (Perera *et al*., 2013). This is attributed to the fact that dogs in these regions are rarely

treated for parasitic diseases and policies on pet ownership are usually lacking (Ugbomoiko *et al*., 2008) or poorly enforced, thereby providing fertile grounds for zoonotic transmission of parasites. It is therefore important to understand the epidemiology of helminth infections in dogs to improve animal health and prevent the spread of zoonotic pathogens to humans. This study was aimed to estimate the prevalence of GIT parasites of dogs and assess the awareness of the community about dog parasitic zoonoses in Chagni town, northwest Ethiopia.

Materials and methods

Study Area

The study was conducted in Chagni (formerly called "Kedamawi Haile Selassie Ber") town in northwestern Ethiopia. Located in the Awi Zone of the Amhara National Regional State, this town has a longitude and latitude of 10°57′N 36°30′E and an elevation of 1583 meters above sea level. It is the administrative center of Guangua district (woreda) (Fig 1.). Based on the figures from the Central Statistical Agency 2005, Chagni has an estimated total population of 30,938, of whom 16,035 are men and 14,903 are women (CSA, 2005).

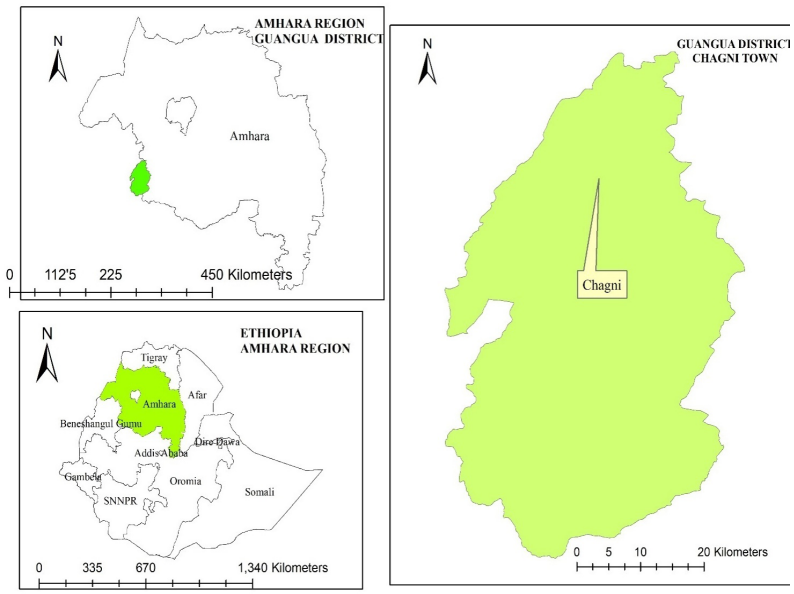


Figure 1: Map of the study area

Study animals and study design

The study was conducted on stray dogs at chagni town using cross-sectional study design. Sample size was determined based on studies conducted previously (Amissah-Reynolds *et al*., 2016; Idika *et al*., 2017; Kiflu *et al*., 2016; Israel, 1992). Fecal sample collection and questionnaire were the methods used.

Fecal sampling

Fecal samples were collected from 202 stray dogs. In order to avoid repeat sampling of the same dog, sample collection was done in the morning in the places where dogs overnight and in different places at the same time as well as photo of each dog was taken using mobile phones. Furthermore, a number of individuals (trained animal heath technicians were involved to collect samples). Information was obtained on the approximate age, sex, and mode of life of each dog. Fecal samples were placed in clean 30 ml bottles. About 10g of feces from each dog were mixed thoroughly with 10% aqueous formaldehyde for preservation. Samples were examined for parasite eggs in the laboratory by means of the modified Kato–Katz procedure (Hendrix, 2003). Each parasite egg was identified using established structural and morphometric criteria (Soulsby, 1982).

Postmortem examination

This was conducted on 9 stray dogs euthanized by the municipal in Chagni. An abdominal cut was made in each animal and the intestine was tied from the pyloric and anal ends and collected in a bag. Bags were stored in an icebox and carried to the laboratory within 3 hours. The carcasses were burned in the field to ensure that there was no contamination of the environment. In the laboratory, each intestine was divided into four pieces of equal length. Each piece was cut longitudinally and soaked in 0.15 M phosphate buffer saline (PBS, pH 7.2) for 5 min. The mucosal lining was gently scraped with a spatula into clean glass dishes and the collected intestinal contents were left to settle in1000ml conical Nalgene graduates (Nalge, Rochester, USA). Following several washes with PBS, aliquots were examined under a dissecting microscope. The preparation, staining and mounting of helminths were carried out as described by Meyer and Olsen (1980). Identification of intestinal helminths was based on keys and descriptions by Khalil *et al*. (1994). Identification of various *Taenia* species was based on stained specimens and comparison of scoleces and

various types of proglottids as well as on morphometric measurements of small and large hooks of armed rostella (Soulsby, 1982).

Questionnaire survey

A total of 82 questionnaires were administered to household members of the community in Chagni town. A structured questionnaire was prepared in local language and a pilot test to validate the questionnaire was performed. The questionnaire was designed to gather information on dog ownership, feeding of dogs, treatment for dogs, the extent of awareness on dog parasites, control measures taken, the occupation of the dog's owner and other related factors. The participants were selected based on simple random sampling from the lists of kebeles in the town.

Statistical analysis

Statistical tests were performed using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA). The chi-square (χ^2) test was used to assess difference in frequency of the helminthes between the sex and age groups. In all cases, 95% confidence intervals and $P < 0.05$, were set for significance.

Results

Prevalence and species of gastrointestinal parasites in stray dogs

The overall infestation of dogs with gastrointestinal parasites was 94.6% and 100% based on fecal examination and postmortem examination, respectively. Gastrointestinal helminth parasites of dogs identified based on fecal examination were: *Taenia spp, Ancylostoma* spp*., Tirchuris vulpis, Toxocara canis, Dipylidium caninum*, *Strongyloides stercoralis* and protozoa spp(Table 1). Infection with only one species of parasite was more common (35.6%) than infection with two (28.7%), three (20.3%) or four (9.9%) species. Overall frequency of infection with GI helminthes in dogs and frequency in different sex and age groups obtained by fecal examination was described in Table 2. Statistically significant variation was seen between age groups ($P < 0.05$).

Table 1: Intestinal parasites diagnosed in the faeces of dogs and their respective prevalence in Chagni, northwest Ethiopia

Parasite	No. (%) Positive
Taenia spp	191(94.6)
Ancylostoma spp	187(92.6)
Tirchuris vulpis	178(88.1)
Toxocara canis	177(88.0)
Dipylidium caninum	124(61.4)
Strongyloidesstercoralis	122(60.1)
Protozoa spp	113(55.9)

Out of 9 male adult dogs examined at necropsy, all of them (100%) harbored one or more GI helminthes. Of these, 77.8% were infected with two or more GI helminthes. Based on postmortem examination dog gastrointestinal helminthes identified were: *Echinococcus granulosus, Taenia hydatigena, Taenia ovis, Ancylostoma caninum, Tirchuris vulpis, Toxocara canis* and *Dipylidium caninum*. The study revealed that *Taenia* spp. was more prevalent followed by nematodes (Table 3). The dogs used for postmortem examination were adult (age greater than one year) males, no comparison was made between sex and age groups.

Table 2: Overall frequency of infection with GI helminthes in dogs and frequency in different sex and age groups obtained by coproscopical examination

Sex of dogs	No. examined	No. (%) infected	X2(P-value)
Male	121	117(96.7)	0.113
Female	81	74(91.4)	(P >0.05)
Total	202	191(94.6)	
Age of dogs (Months)			
0-6	24	17(70.8)	7.6
7-11	63	60(95.2)	(P <0.05)
≥ 12	115	114(99.1)	
Total	202	191(94.6)	

Table 3: Intestinal parasites of 9 adult male dogs identified based on postmortem examination in Chagni, northwest Ethiopia

Parasite	No. (%) Positive
Echinococcus granulosus	8(88.9)
Taenia hydatigena	6(66.7)
Taenia ovis	4(44.4)
Ancylostoma caninum	7(77.8)
Tirchuris vulpis	7(77.8)
Toxocara canis	8(88.9)
Dipylidium caninum	6(66.7)
Mesocestodes	9(100)

Community awareness on zoonoses

Of the community members, 68.3% reported that tapeworms (*D. caninum,* and hydatids (*E. granulosus*) locally called "kosso" were zoonotic agents, followed by roundworms (*Toxocara*, locally name "wosefat"). Of the owners who were aware of the potential for transmission of parasites from dogs to humans, none of them could provide correct information on the mode of transmission. Of the dog owners 50(61%) had not treated their dogs using anthelmintics in their life time where as 39% treated their dogs using traditional preparations. Majority of the community (65.9%) members reported feeding to dogs' raw condemned offals and human food and 61% reported usual place for defecation of dogs is on the street (Table 4).

Table 4: knowledge and attitudes of dog owners regarding potential zoonotic disease in the traditional communities of Chagni, northwestern Ethiopia

Variables	No (%)
Dog ownership	
One dog	50(61)
Two or more dogs	32(39)
Occupation	
Civil servant	20(24.4)
Marchant	62(75.6)
Reasons for keeping dog	
Hunting	0
Look after livestock	41(51.2)
No specific reason	20(24.4)
Look after the house at night	20(24.4)
Housing of dogs	
Confined to dog house on compound	32(39)
Share the same house with the owners and livestock	50(61)
Feeding of dogs	
Condemned offals	28(34.1)
Condemned offals and human food	54(65.9)
Usual place of defecation of dogs	
Within the house premises	32(39)
On the street	50(61)
Do children play with dogs	
Yes	74(90.2)
No	8(9.8)
Community awareness of diseases transmitted by dogs	
Serious	18(21.9)
Not serious	33(40.2)
Do not cause any disease	31(37.9)
What are the main parasitic diseases of dogs	
Taenia species ("Kosso" locally)	56(68.3)
Toxocaracanis ("wosefat")	26(31.7)
Treatment for dogs	
Not at all	50(61)
Use traditional preparations	32(39)

Discussion

The present study provides quantitative estimates of parasites in dogs in Chagni town, northwest Ethiopia. The results showed that gastrointestinal helminth species were abundant, and that prevalence of infection was very high whereas the awareness of the community about zoonotic diseases transmitted by pets was insufficient.

The parasites reported in this study have been previously documented in dogs throughout the world (Senlik*et al*., 2006), with a pronounced difference in prevalence and density between regions. This discrepancy in the overall frequency rates reported in various countries could be attributed to the difference in health care and animal management practices followed in different countries.In our study gastrointestinal helminth infestation of dogs was found to be94.6% and 100% based onfecal examination and postmortem examinations, respectively. There was no significant difference in the results obtained between the two techniques. The slight difference observed could be due to the fact that fecal examinations may not detect the immature parasites, which are unable to lay eggs. However, the overall frequency rate thus obtained on fecal examination is in agreement with previous works inTurkey (Senlik *et al.,* 2006), Netherlands (Nobel *et al.,* 2004), and in Greece (Haralabidis *et al*., 1988) but a lower frequency rate was recorded in Santa Catarina (Blazius *et al*., 2005) and in Nigeria (Ugochukwu and Ejimadu, 1985).

The difference in the frequency of the nematode infections between countries is possibly due to the differences in climatic factors required for the biology of the parasites, veterinary facilities and public awareness to take care of the dogs. During the survey it was noted that large number of dogs scavenge at abattoirs and at butcher shops and those kept indoors are also frequently fed uncooked offal that are not in good hygienic condition. It is also common to find animal cadaver thrown into street where dogs communally feed on, which could be a suitable media for transmission of the parasites.

This potential for human zoonotic disease has rarely been addressed in control programs in Ethiopia and other low-income countries (Merga and Sibhat, 2015). Considering the high prevalence of gastrointestinal helminth infections found in dogs, and the close bonds in which dogs live together with people, the risk of transmission of these parasites to humans seems to be obvious. For

example, *Toxocara* infection in humans may cause visceral larva migrans, in severe cases leading to blindness (Taylor, 2001), and dog hookworm infections put humans at risk for *cutaneous larva migrans* which is endemic in many resource-poor communities (Heukelbach *et al.,* 2005).

The prevalence reported in this study differs from those of Hailu *et al*., (2007) who recorded 51% based on fecal examination and nearly similar result on postmortem examination. The recorded high prevalence of gastrointestinal parasites of dogs in Chagni town may be due to poor awareness of the community. Our data shows that most dogs never or rarely received antiparasitic treatment during their puppy stage, and only few people were aware of the zoonotic potential of dog parasites.

The diagnostic technique of parasites done in this study, based on the morphological characteristics of ova under light microscope, has the disadvantage that it fails to distinguish *E. granulosus* from other Taenidae. Thus, *E. granulosus*, a major zoonotic parasite of livestock and dogs in Ethiopia (Kebede *et al*., 2009) may be highly prevalent as it is indicated in the postmortem examination. The fact that dogs enjoy unrestrained association with humans, scavenge for food in an environment contaminated with faecal material of potential intermediate hosts and feed on offal of slaughtered livestock in abattoirs (Jones *et al*., 2012) makes transmission of zoonotic parasitic diseases predictable in the setting studied.

In general, the trend in prevalence and species composition of parasites observed in this study may reflect the degree of environmental contamination in particular, *T. canis, A. caninum* and *D. caninum* are zoonotic parasites constituting public health problems in the study areas.

In conclusion, the study shows the presences of different nematode species in a single host as well as high frequency of these parasites in the study area which warrants serious attention due to pathogenic impact of the parasites. In addition, parasites of importance for human health were highly prevalent in Chagni area dogs and that intervention measures are necessary to reduce the risk of transmission of parasites from dogs to humans. Interventions should focus on health education provided to dog owners, strategic deworming of dogs using broad-spectrum anthelmintics and the establishment of a program based

on zoonotic diseases, have paramount importance. Moreover, further epidemiological studies should be conducted seasonally in different regions of the country.

Acknowledgements

This work was financially supported by Addis Ababa University. W/ro Wolela Guade and Ato Mekuriaw Tessema are acknowledged for sample collection at Chagni.

References

Abdi, J., Asadolahi,K., Maleki, M. H. and Hafez, A. A., 2013. Prevalence of helminthes infection of stray dogs in Ilam province. *J. Paramedical Sci.*, 4(2),47–50.

Abere, T., Bogale, B. and Melaku, A., 2013. Gastrointestinal helminth parasites of pet and stray dogs as a potential risk for human health in Bahir Dar town, northwestern Ethiopia. *Vet World* 6(7),388-392.

Abo-Shehada, M. N., 1989. Prevalence of Toxocara ova in some schools and public grounds in northern and central Jordan. *Ann. Trop. Med. Parasitol*., 83, 73-75.

Ahn, S.J., Ryoo, N.K., Woo, S.J., 2014. Ocular toxocariasis: clinical features, diagnosis, treatment, and prevention. *Asia Pac. Allergy* 4(3), 134–41.

Amissah-Reynolds, P.K., Monney, I., Adowah, L.M., andAgyemang, S.O., 2016. Prevalence of Helminths in Dogs and Owners' Awareness of Zoonotic Diseases in Mampong, Ashanti, Ghana. *J. Parasitol. Res*., Article ID 1715924, 6 pages. dx.doi.org/10.1155/2016/1715924

Ayinmode, A.B., Obebe, O.O., and ,Olayemi, E. 2016. Prevalence of potentially zoonotic gastrointestinal parasites in canine faeces in Ibadan, Nigeria. *Ghana Med. J*., 50(4), 201–206.

Blazius, R.D., Emerick, S., Prophiro, J, S, Romao, P.R. and Silva, O.S., 2005. Occurrence of protozoa and helminths in fecal samples of stray dogs from Itapema City Santa Catarina. *Rev. Soc. Bras. Med. Trop*., 38(1), 73-74.

Chomel, B.B.,2014. Emerging and re-emerging zoonoses of dogs and cats. *Animals (Basel)*4(4), 434–445.

Croese, J., Loukas, A., Opdebeeck, J., Prociv, P., 1994. Occult enteric infection by *Ancylostoma caninum*: a previously unrecognized zoonosis. *Gastroenterol*.,106 (1), 3–12.

CSA (2005). Central Statistical Agency of Ethiopia. Annual report.

Cui, J. and Wang, Z.Q., 2001. Outbreaks of human trichinellosis caused by consumption of dog meat in China. Parasite, 8(2 Suppl), S74–S77.

Dubná, S., Langrová. I., Jankovská, I., Vadlejch, J, Pekár, S., Nápravník, J, *Fechtner, J.,2007.*Contamination of soil with *Toxocara* eggs in urban (Prague) and rural areas in the Czech Republic. *Vet. Parasitol*., 144, 81–6.

Gaunt, M. C. and Carr, A. P., 2011. A survey of intestinal parasites in dogs from Saskatoon, Saskatchewan. *Canadian Vet. J.*,52(5), 497–500.

Habluetzel, A., Traldi. G., Ruggieri. S., Attili. A., Scuppa. P., Marchetti. R., Menghini, G., Esposito, F., 2003. An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. *Vet Parasitol.*,113, 243–252.

Hailu, Y., Ayele, T., Fikru, R. and Basu, A.K., 2007. Gastrointestinal nematodes in dogs from Debre Zeit, Ethiopia. *Vet. Parasitol.,* 148, 144-148.

Haralabidis, S.T, Papazachariadou, M.G., Koutinas, A.F. and Rallis, T.S.,1988. A survey on the prevalence of gastrointestinal parasites of dogs in the area of Thessaloniki, Greece. *J. Helminthol*., 62(1), 45-49.

Hendrix, C.M., 2003. Laboratory Procedures for Veterinary Technicians. 4th edition, Mosby, Inc., USA, pp364.

Heukelbach, J., Walton, S.F., Feldmeier, H., 2005. Ectoparasitic infestations. *Curr. Infect. Dis. Rep.,* 7(5), 373-80.

Idika, I.K., Onuorah, E.C., Obi, C.F., Umeakuana, P.C., Nwosu, C.O., Onah, D.N., and Chiejina, S.N., 2017. Prevalence of gastrointestinal helminth infections of dog in Enugu State, South Eastern Nigeria. *Parasite Epidemiol Control*, 2(3): 97–104.

Israel G. D., 1992. Sampling the evidence of extension program impact. Program Evaluation and Organizational Development, IFAS, University of Florida. PEOD-5.

Joffe, D., Van Niekerk, D., Gagne, F., Gilleard, J., Kutz, S. and Lobingier, R., 2011.The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta. *Can. Vet. J*., 52(12), 1323–1328.

Johnson, S. A. M., Gakuya, D. W., Mbuthia, P. G., Mande, J. D., and Maingi, N., 2015. Prevalence of gastrointestinal helminths and management practices for dogs in the Greater Accra region of Ghana. *Heliyon*, 1(1), Article ID e00023.

Jones, O., Kebede, N., Kassa, T.,Tilahun. G., Macias, C., 2012. Occurrence of bovine hydatidosis and evaluation of its risk to humans in traditional communities of Southern Region of Ethiopia. *Ethiop. J. Health Dev.,* 26(1), 43-48.

Kebede, N., Mitiku, A. and Tilahun, G., 2009. Hydatidosis of slaughtered animals in Bahir Dar Abattoir, Northwestern, Ethiopia. *Trop. Anim. Hlth. Prod.,* 41, 43-50.

Khalil, L.F., Jones, A., Bray, R.A., 1994. Keys of the cestode parasites of vertebrates. CAB International, Wallingford, UK, pp 751.

Kiflu, B., Abdurahaman, M., Alemayehu, H., and Eguale, T., 2016. Investigation on public knowledge, attitude and practices related to pet management and zoonotic canine diseases in Addis Ababa, Ethiopia. *Ethiop. Vet. J*., 20 (1), 67-78.

MacPherson, C.N.L., Meslin, F.X., Wandeler, A.I., 2000. Dogs, zoonoses and public health. Oxon, New York: CABI Publishing; pp. 213–56.

Merga, T., and Sibhat, B., 2015. Prevalence of gastrointestinal helminth parasites of dogs and associated risk factors in Adama Town, Central Ethiopia. *Ethiop. Vet. J.,* 19 (2), 91-103.

Meyer, M.C. and Olsen, O.W., 1980. Essentials of parasitology. 5th edition, Brown, Du-bu-due, Iowa, USA, pp 298.

Mirazaei, M., andFooladi,M., 2012. The prevalence of intestinal helminths in owned dogs in Kerman city, Iran. *Asian Pacific J. Trop. Med.,* 13(1), 735-737.

Nobel, W.E., Robben, S.R., Dopfer, D., Hendrix, W.M., Boersema, J.H., Fransen, F. and Eysker, M., 2004. Infections with endoparasites in dogs in Dutch animal shelters. *Tijdschr. Diergeneeskd.*, 129(2), 40-44.

Oliveira-Sequeira, T.C. Amarante, A.F. Ferrari, T.B. and Nunes. L.C., 2002. Prevalence of intestinal parasites in dogs from Sao Paulo State, Brazil. *Vet. Parasitol*., 103(1-2), 19-27.

Paul, M. King,L. and Carlin,E. P.,2010. Zoonoses of people and their pets: a US perspective on significant pet-associated parasitic diseases. *Trends in Parasitol.*, 26(4), 153–154.

Paulos, D., Addis, M., Fromsa, A. and Mekibib, B., 2012. Prevalence of gastrointestinal helminthes among dogs and owners perception about zoonotic dog parasites in Hawassa Town, Ethiopia. *J. Pub. Hlth. Epid.,* 4(8), 205-209.

Perera, P.K., R. P. V. J. Rajapakse, and Rajakaruna, R. S., 2013. Gastrointestinal parasites of dogs in Hantana area in the Kandy District. *J. National Sci. Found. Sri Lanka*, 41(2), 81–91.

Robertson, I.D., Irwin P.J., Lymbery, A.J., Thompson R.C.A., 2000. The role of companion animals in the emergence of parasitic disease. *Int. J. Parasitol*., 30(12-13), 1369–1377.

Rubel, D., Wisnivesky, C., 2005. Magnitude and distribution of canine fecal contamination and helminth eggs in two areas of different urban structure, greater Buenos Aires, Argentina. *Vet Parasitol., 133, 339–47.*

Senlik, B., Cirak, V.Y. and Karabacak, A., 2006. Intestinal nematode infections in Turkish military dogs with special reference to Toxocaracanis. *J. Helminthol.,* 80, 299-303.

Soulsby, E.J.L., 1982. Helminthes, arthropods and protozoa of domesticated animals. The English Language Book Society and Bailliere Tindall, London, UK, pp809.

Tannen, D. 2004. Talking the dog: framing pets as interactional resources in family discourse. *Res. Lang. Soc. Interac.*, 37, 399- 420.

Taylor, M.R. 2001. The epidemiology of ocular toxocariasis. *J. Helminthol.*, 75(2), 109-18.

Ugbomoiko, U. S., Ariza, L. and Heukelbach, J., 2008. Parasites of importance for human health in Nigerian dogs: high prevalence and limited knowledge of pet owners. *BMC. Vet. Res.*,4, article 49.

Ugochukwu, E.I. and Ejimadu, K.N., 1985. Studies on the prevalence of gastro-intestinal helminths of dogs in Calabar, Nigeria. *Int. J. Zoonoses*, 12 (3), 214-218.

Walker, N.I., Croese. J., Clouston. A.D., Parry. M., Loukas. A., Prociv, P., 1995. Eosinophilic enteritis in northeastern Australia. Pathology, association with *Ancylostoma caninum,* and implications. *Am. J. Surg. Pathol., 19, 328–37.*

Helminth infection of layer and dual-purpose chickens kept under small scale intensive production system, Ethiopia

Meskerem Adamu[1], Kasech Melese[2], Mekonnen Germa[3], Tewodros Fekadu[2] and Seid Ali[2]*

[1] Ethiopian Biotechnology Institute, Addis Ababa, Ethiopia

[2]Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia

[3]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia

**Corresponding author: Meskerem Adamu, madchere@gmail.com or mesku12345@yahoo.com*

Abstract

Losses due to reduced productivity caused by helminthiasis are economically important to the poultry industry. There are a few research reports in the prevalence of helminth-parasites in the different poultry production systems. A cross sectional study was conducted with the aim of estimating the prevalence of helminth infections in exotic layer and dual-purpose chickens, kept under small scale intensive farms of selected towns, Ethiopia: Addis Ababa (Highlands) and Debre Zeit, Haramaya, Hawassa and Kombolcha (Midlands) climatic zones. Towns and farms were purposively identified while chickens from each farm were randomly selected for fecal sample collection and necropsy examination. A total of 1009 fresh fecal samples and thirty-three chickens were collected from chickens of different breeds. Helminth egg and adult parasite identification was processed through coproscopic and necropsy examination methods, respectively. The overall prevalence of helminth infection was 66(%). A statistically significant difference was found in the prevalence of helminthiasis among study sites ($P<0.01$) and between chicken breeds ($P<0.02$). Helminthiasis was relatively higher in Addis Ababa (72%), Debre Zeit (71%) and Haramaya (67%) compared to Hawassa (56%) and Kombolcha (34%). Helminth infection in Dominant Sussex was higher, 90(%), followed by White Leghorn chicken 80(%), Bovans 67(%), Koekock 58(%), Dominant Red Barred 57(%), Lohman Brown 42(%), Fayoumi 40(%) and Lohman Dual 33(%). However, the prevalence did not vary ($P>0.05$) among age groups. Three species of nematodes as *Ascaridia galli* (52%), *Heterakis gallinarum* (57%) and *Capillaria* species (1%) and one species of cestode: *Raillietina* species (3%) were identi-

fied. Regular check up for helminth infection and application of appropriate prevention and control measures are important.

Key words: Chicken; Exotic; Intensive; Helminth-infection; Small-scale

Introduction

There are approximately 25–30 medium to large scale integrated poultry farms in Ethiopia, that keep, process and distribute their products by themselves. Large scale layer farmers rear pullets themselves, mostly from their own or imported day old chicks. However, some medium and most small-scale farmers either choose for pullet rearing or for layer keeping (Boere *et al.,* 2015). There is a gradual increase in commercial small- and medium-scale chicken production in the country (Pagani and Wossene, 2008). In the small-scale intensive system of production in urban and peri-urban areas of the country, small number of exotic breeds of chickens are produced using relatively modern management methods. This activity is being undertaken as a source of income in and around major cities of the country. These farms are involved in the production and supply of table eggs to various supermarkets, kiosks and small roadside restaurants through middlemen (Demeke, 2008). However, most of the poultry (meat and egg) products consumed still come from backyard village poultry systems (Dessie and Ogle, 2001; Udo *et al.,* 2006; Boere *et al.,* 2015). In general, the contribution of the chicken industry to the national economy and per-capita meat and egg consumption is very low. Several factors have been suggested for the low production and productivity of chickens. Poultry diseases continue to play a major role in directly interfering with poultry productivity, which decreases economic returns and may therefore negatively affect the development of the industry. Losses due to reduced productivity (increased feed conversion ratio, poor weight gain, poor egg production, death of young birds) caused by helminthiasis are economically very important to the poultry industry. There are several types of helminths that cause a serious economic problem to poultry industry. Intestinal worms, large roundworm, hairworms, threadworms, but the most common is the large roundworm (*Ascaridia galli). Ascaridia galli* is a nematode occurring in the small intestine of chickens. It is a worldwide problem in all poultry production systems, especially in free-ranging birds and is a cause of economic loss in modern poultry. Light to medium infestations may not produce clinical signs; however, heavy infestations may cause diarrhea, intestinal occlusion, intussusceptions, emaciation, anemia, reduced egg production and death. This parasite has been implicated in some cases of egg

yolk peritonitis. *Heterakis gallinarum* found in the lumen of the ceca**.** Infection with *H. gallinarum* may not show clinical signs but is capable of transferring the protozoa *Histomonas meleagridis* to chickens, a species of protozoan parasite that causes histomoniasis or blackhead disease. *Capillaria* species are located throughout the intestinal tract. Birds are infected when ingesting the earth worms. Infections with *Capillaria* spp. can be highly pathogenic for birds kept in deep-litter systems where big numbers of infective eggs may build up in the litter or in the soil. *Raillietina* is a cestode and located in the small intestine where the scolex is embedded into the mucosa. Chronic infections are characterized by reduced growth, emaciation and weakness (Merck, 2011; PDHA, 2009; Permin and Hansen, 1998). A number of endo-parasites are widely studied and reported in traditional systems of poultry production throughout the world including Ethiopia (Ashenafi and Eshetu, 2004). The prevalence of parasitic diseases in poultry seems to have been reduced significantly in commercial indoor poultry production systems due to improved housing, hygiene and management. However, parasitic diseases continue to be of great importance in deep-litter and free-range commercial systems (Merck, 2011). Besides, there are only a few reports on the prevalence and significance of endo-parasites in the different poultry production systems (Permin and Hansen, 1998). Research in poultry diseases and parasites in the different poultry production systems of the country is limited. Therefore, the study was designed to determine the occurrence of helminthiasis in exotic *layer* and dual-purpose chickens kept under small scale intensive management system of the selected towns of Ethiopia.

Materials and methods

Study area and study design

A cross sectional study was conducted between 2015 and 2016 to determine the occurrence of helminthiasis circulating in small scale *layer* and dual-purpose chicken farms, under intensive deep-litter management system of 5 selected towns, Ethiopia: Addis Ababa, Debre Zeit, Kombolcha, Haramaya and Hawassa. Addis Ababa has a subtropical highland climate with an altitude of 2,355 meters above sea level. The average annual rainfall and average maximum and minimum temperature for the area are 1,165mm, and 30.6 ^{0}C and 0 ^{0}C, respectively. Debre Zeit is located 47.9 km south east of Addis Ababa, the town is located in the east Shewa Zone of the Oromia Region and has a midland climate with an altitude of 1,920 meters above sea

level. The average annual rainfall and average maximum and minimum temperature for the area are 800mm, and 27.7 ^{0}C and 12.3 ^{0}C respectively. Kombolcha is a city and woreda in north-central Ethiopia, located in the Debub Wollo Zone of the Amhara Region and has a midland climate with an elevation between 1842 and 1915 meters above sea level. Haramaya is a town located in the East Hararghe Zone of the Oromia Region, located in the eastern part of Ethiopia and has a midland climate with an altitude of 2,047 meters above sea level. Hawassa is a city located in the Southern Nations, Nationalities, and Peoples' Region. It is located 273 km south of Addis Ababa and has a midland climate with an altitude of 1708 meters above sea level.

Sampling technique

Because of the selected towns are very well-known for their small scale intensive deep-litter chicken rearing practices and to have relatively high density of chickens, purposive sampling method was been carried out to identify towns while random sampling method was used to identify chickens per farm. A total of 1009 fresh fecal samples from individual layer and dual-purpose chickens and thirty-three apparently healthy chickens were used for fecal sample collection and necropsy examination, respectively. Chickens were selected according to their age groups and breed types. The age was conveniently subdivided into young growers from 2-9 months and into adult chickens to those of 10 and greater than 10 months. Chicken samples were collected from different breeds of layers: Bovans from Addis Ababa (250), Debre Zeit (408) and Kombolcha (93) and White Leghorn (100) chickens and dual purpose: Dominant Red Barred (28), Dominant Sussex (20), Fayoumi (50), Koekock (19), Lohman Brown (26), and Lohman Dual chickens (15).

Sample collection and laboratory procedures

Fecal samples were collected, properly labeled with the necessary information's, placed in clean universal bottle and were kept in portable refrigerator. Then samples were immediately transported to Debre Zeit agricultural research center laboratory, for further laboratory examination. Helminths egg identification was processed by centrifugation flotation method, that involves sodium chloride solution as a floatation fluid. Helminth eggs were identified by their size, shape and the color of the shell wall. Concentration McMaster technique was used for the counting of eggs, that 15 ml of the fecal suspension correspond to 1.0 gram of feces (Permin and Hansen, 1998). The number of eggs per gram of feces (EPG) was calculated by counting eggs in both fields and

by multiplying them by 20 (Permin and Hansen, 1998). The collected chickens were also monitored through necropsy. Incision was made through the cloaca in the midline in cranial direction to inspect the whole abdominal cavity. Gastrointestinal tract: the oesophagus, crop, proventriculus, ventriculus (gizzard) and the whole intestine were placed in the tray and were opened in a longitudinal section and examined. The intestinal contents are carefully washed through a test sieve and the mucosa was carefully scraped and the contents of the sieve transferred to a petri dish and were examined under a stereo microscope through grouping and count of helminths (Kaufmann, 1996; Urquhart *et al*., 1996; Permin and Hansen, 1998).

Data analysis

Collected data were coded and recorded in Microsoft excel spread sheet. Then analyzed using Statistical Package for Social Sciences (SPSS) version 23. Descriptive statistical analysis (mean and percentage) was used to summarize and present the data. Chi-square test was used to evaluate association of chickens breed and age with the prevalence of helminth infection. P-value <0.05 was considered significant.

Results

Out of one thousand nine examined small scale, layer and dual-purpose chickens, the overall prevalence of gastro-intestinal helminths was found to be 66%. A statistically significant ($P<0.01$) difference was found in the prevalence of helminth infection among study sites (Table 1).

Table 1. Overall prevalence of GIT helminth-parasites among chickens of different study sites.

Study sites	Elevation	Climatic zones	No. sampled	No. positive	Prevalence (%)	95% CI
Addis Ababa	2355	Highlands	250	181	72	0.05
Debre Zeit	1920	Midlands	408	289	71	0.04
Haromaya	1708	Midlands	150	100	67	0.07
Hawassa	2047	Midlands	108	68	56	0.09
Kombolcha	1842	Midlands	93	32	34	0.09
Total			1009	669	66	
F						14.2
P-value						<0.001

There was a statistically significant (P<0.02) difference in the prevalence of helminth infection between chickens of different breeds (Table 2).

Table 2. Prevalence of GIT helminth infection among chickens of different breed.

Breed	Study area	No. sampled	No. positive	Prevalence (%)	95% CI
Bovans	Addis Ababa	250	181	72	0.05
Bovans	Debre Zeit	408	289	71	0.04
Bovans	Kombolcha	93	32	34	0.09
	Total	751	501	67	
Dominant Red Barred	Hawassa	28	16	57	1.17
Dominant Sussex	Hawassa	20	18	90	1.13
Fayoumi	Haramaya	50	20	40	0.14
Koekock	Hawassa	19	11	58	4.5
Lohman Brown	Hawassa	26	11	42	3.4
Lohman Dual	Hawassa	15	5	33	0.26
White Leghorn	Haramaya	100	80	80	0.07
	Total	1009	662	66	
F				4.5	
P-value				0.002	

The prevalence of *A. galli, H. gallinarium, Capillaria spp* and *Raillietina spp* of layer and dual- purpose intensively managed chickens was 54, 61, 1.2 and 2.9 (%) in Debre Zeit, 68,70, 1.2 and 2 (%) in Addis Ababa, 45, 49, 2 and 1.3 (%) in Haramaya, 37, 47, 0 and 4.6 (%) in Hawassa and 32, 32, 4 and 4 (%) in Kombolcha, respectively. The prevalence of *H. gallinarium* infection among the study sites was statistically (P<0.01) significant (Table 3).

Table 3. Prevalence of GIT helminth species on the basis of study site.

Study sites	A. galli				H. gallinarium			Capillaria spp			Raillietina spp		
	No. sampled	No. positive	Prevalence (%)	95%CI	No. positive	Prevalence (%)	95%CI	No. positive	Prevalence (%)	95%CI	No. positive	Prevalence (%)	95%CI
AA	250	170	68	0.05	175	70	0.05	3	1	0.01	5	2	0.01
DZ	408	222	54	0.04	250	61	0.04	5	1	0.01	12	3	0.01
HAR	150	67	45	0.08	73	49	0.08	3	2	0.02	2	1	0.01
HAW	108	40	37	0.09	51	47	0.09	0	0	0.00	5	5	0.04
KOM	93	30	32	0.09	30	32	0.09	4	4	0.04	4	4	0.04
Total	1009	529	52		579	57			1			3	
F		14.1			13			1.8			0.9		
P-value		2.8			0.000			0.1			0.4		

AA=Addis Ababa, DZ=Debre Zeit, HAR=Haramaya, HAW=Hawassa, KOM=Kombolcha

Three species of nematodes were identified. The overall prevalence of *A. galli, H. gallinarium, Capillaria* spp and *Raillietina* spp was 52, 57, 1 and 3 (%), respectively. A statistically significant (P<0.01) difference was found in the prevalence of *H. gallinarium* infection among the study breeds (Table 4).

The overall mean EPG count of gastro-intestinal helminthes: *A. galli* ranges from 7 to 364, *H. gallinarium* from 31 to 538, *Capillaria* spp from 0 to 3 and *Raillietina* spp from 0 to 7. Except the *Raillietina* spp, the mean EPG prevalence of nematodes did not vary (P>0.05) among study sites (Table 5).

Table 4. Prevalence of GIT helminth-parasite species on the basis of breed.

Study sites	A. galli				H. gallinarium			Capillaria spp			Raillietina spp		
	No. sampled	No. positive	Prevalence (%)	95%CI	No. positive	Prevalence (%)	95%CI	No. positive	Prevalence (%)	95%CI	No. positive	Prevalence (%)	95%CI
BOV	751	415	55	0.06	455	61	0.06	12	2	0.2	21	3	0.2
DRB	28	12	43	0.19	15	54	0.2	0	00	0.0	0	00	0.0
DS	20	11	55	0.2	17	85	0.2	0	00	0.0	2	10	0.02
FAY	50	17	34	0.1	12	24	0.1	2	4	0.2	2	4	0.01
KK	19	3	16	0.2	5	26	0.2	0	00	0.0	3	16	0.02
LB	26	10	38	0.2	9	35	0.2	0	00	0.0	0	00	00
LD	15	4	27	0.3	5	33	0.3	0	00	0.0	0	00	00
WLH	100	50	50	0.09	.61	61	0.09	1	1	0.2	0	00	00
F		0.1			5.1			1.8			0.9		
P-value		1.9			0.000			0.1			0.4		

Table 5. Mean EPG count SE of helminth-parasites on the basis of study sites.

Study sites	A. galli			H. gallinarium			Capillaria spp			Raillietina spp		
	Mean	Standard Error	95%CI	Mean	Standard Error	95%CI	Mean	Standard Error	95%CI	Mean	Standard Error	95%CI
AA	125	8.9	17.5	538	44.1	86.9	2.60	1.5	2.9	0.02	0.0	0.01
DZ	364	14.9	29.3	484	19.1	37.5	0.00	0.0	0.0	10.6	3.4	6.7
HAR	84	10.4	20.7	363	37.2	73.6	0.54	0.3	0.7	0.01	0.0	0.01
HAW	65	13	25.7	126	14.7	29.3	1.78	0.6	1.2	0.03	0.02	0.03
KOM	7	1.1	2.36	31	5.8	11.5	3.10	1.6	3.1	2.4	1.2	2.4
F	106			33			2.2			3.2		
P-value	6.3			4.4			0.06			0.01		

EPG=egg count per gram of feces; SE= Standard Error

AA=Addis Ababa, DZ=Debre Zeit, HAR=Haramaya, HAW=Hawassa, KOM=Kombolcha

Bovans chicken, collected from around Debre Zeit and Addis Ababa showed a sever *A. galli* infection (Figure 1).



Figure 1. Small intestine of layer chickens is showing a large number of adult and larva *A. galli* worms

Discussion

In the current study the overall prevalence of GIT helminth infections in small scale chicken farms under intensive deep-litter management system was 66%. This was lower compared to that of 79.9%, reported in small scale commercial chickens of Bahir Dar town by Belete and Addis (2015) and 98.9%, reported in broiler breeders of the United States by Yazwinski *et al* (2013). The prevalence of helminthiasis was relatively higher in Central Ethiopia: Addis Ababa (highlands) and Debre Zeit (midlands) and Haramaya (midlands) than those of Kombolcha and Hawassa (midlands) climatic zones. According to the findings reported by Ashenafi and Eshetu (2004), there was a significant difference in the prevalence rate of helminth parasites between the different agro-climatic zones. However, the difference found among the prevalence's of the current study areas couldn't be related to the different climatic zones. Most probably it could be due to the management practices of those areas. The increased intestinal helminth infections reported in Central Ethiopia, could be resulted due to the poor management, health care practices and bio-security status of some small-scale chicken farms. Management problems in general, were common in Central Ethiopia: Debre Zeit and Addis Ababa. (Wossene, 2006). This was confirmed through our direct observation while we were collecting samples, we observed a large number of chicken houses, with poor construction systems, close to each other, with chickens of different production type; either

broiler or layer and with chickens of different age and breeds. The presence of old build up used litters, droppings, extensively reared endogenous chickens, domestic animals and family houses also observed, very close to the majority of chicken houses. This was in line with the report of Wossene (2006), Ayele and Rich (2010) and Adamu (2015), who have also reported that the management, health care practices and bio-security status in many of the small-scale intensive poultry farms in Ethiopia are generally poor. The high prevalence of GI helminth infections among the small scale intensively managed chickens reported in the current study was in consistent with those previously reported from different parts of the world for instance: Yazwinski *et al* (2013) from United States and *Wokem and Obiyor (2018)* from *Nigeria*. This was supported with the discussion reported by Permin and Hansen (1998) and Merck (2011), who described that modern confinement rearing of poultry has significantly reduced the incidence and diversity of endoparasite infections, which are common in ranged birds and in backyard flocks. However, severe parasitism still may be seen in floor-reared chickens where management problems may exist. The 34% of prevalence reported from Kombolcha is consistent with that of Ngongeh *et al* (2014), who reported the prevalence of 34.4% of GI helminths in slaughtered layer chickens reared in Enugu State, Nigeria. According to an informal discussion done with some *layer* chicken farm owners whose chickens were diagnosed with widespread *A. galli* and *H. gallinarium* infection, reported that their chicken flocks were never treated against helminth infection. However, they were properly vaccinated and supplemented with balanced feed but the egg production was reduced up to about 30 percent. This could be due to the chronic and un-treated helminth infections. This was in line with the report of Shane (2005), who discussed that extensive *A. galli* infection may reduce egg production in floor housed breeders and commercial layers. Some poultry farmers also reported that they have no idea about the occurrence of helminth infection in the intensive deep-litter management system. However, whenever management problems exist such as poor biosecurity practices and accumulation of used litter and droppings around chicken house, they may serve as shelter and breeding places for intermediate hosts of helminths like, earthworms, insects, other invertebrates and for the accumulation of infective eggs. As a result, confined chickens can be easily affected by eating the intermediate and/or paratenic hosts which move to the chicken houses containing infective eggs of helminths.

A significant variation was found in the *A. galli* and *H. gallinarium* infection among different chicken breeds of the study areas. The variation of helminth

infection among chicken breeds of the same management system could be due to the genetic resistance variation of the chickens. Helminth infection in layer (White Leghorn and Bovans) and Dominant Sussex (dual- purpose) chickens was higher than the other dual-purpose chickens. This was in line with the report of Kaufmann (2011), who found the higher resistance to *A. galli* infection of Lohmann Tradition and Lohmann Brown hens than that of Lohmann LSL classic and ISA Brown hens when exposed to an experimental *A. galli* infection. Abdelqader *et al* (2007), also reported the higher resistance of local chickens than Lohmann LSL white chickens. Local chickens of Jordan infected with *A. galli* revealed significantly fewer worms and excreted less *A. galli* eggs than those of Lohmann LSL white chickens.

The overall prevalence of GI nematodes *A. galli* and *H. gallinarum* was 52% and 57%, respectively. This was higher compared to those reported by Belete and Addis (2015) who reported the prevalence of 20.9 and 17(%), respectively in small scale commercial chickens of the Bahir Dar town. The reason for the higher nematodes infection is similar to those previously indicated for the higher prevalence of helminthiasis. The most prevalent nematode species identified were *A. galli* and *H. gallinarum*. This result is in agreement with those previously reported in laying hens kept in organic production systems in Germany by Kaufmann (2011), who reported the prevalence of *H. gallinarum* (98 %) and *A. galli* (88 %) as the most prevalent nematode species. The prevalence of *Capillaria* and *Raillietina* species reported in the current study is lower. This is closely related to those reported by Beyene et al (2014), Ngongeh *et al* (2014) and Belete and Addis (2015).

The mean egg count per gram of feces (EPG) are important for monitoring helminth infections at individual as well as population level (Permin and Hansen, 1998). The mean EPG count of Addis Ababa, Debre Zeit and Haramaya showed more severe *H. gallinarium* infection *(EPG=538, 484 and 363),* respectively while chickens of Debre Zeit and Addis Ababa showed more severe *A. galli* infection *(EPG=364 and 125),* respectively compared to the mean egg count of others studied chickens. This was in agreement to those previously reported by *Bachaya et al., 2015*, who reported that *White leghorn (layer) chickens, reared in deep litter had more severe infection of A. galli (EPG=1920) compared with those of battery cages birds (EPG=500).* The mean EPG count of *Raillietina* species was significantly ($P<0.01$) different among the study sites. However, mean EPG count of *A. galli, H. gallinarium* and *Capillaria* did not vary ($P>0.05$) among the study site.

Conclusion

The prevalence of helminth infection among the study chickens was higher. A significant difference was found in the prevalence of helminth infection among study sites and chicken breeds. However, the prevalence did not vary among different age groups. Three species of nematodes as *A. galli*, *H. gallinarum* and *Capillaria* species and one species of cestode: *Raillietina* were identified. *H. gallinarum* and *A. galli* are the most prevalent nematode species found. The mean egg count of Addis Ababa, Debre Zeit and Haramaya showed more severe *H. gallinarium* infection while chickens of Debre Zeit and Addis Ababa showed more severe *A. galli* infection. Proper control and prevention measures through awareness creation, proper sanitation, disposal of droppings and removal of used litters, regular checkup for helminth infection, timely deworming, rearing of chickens separately by their breed management system and age groups, would help to improve egg and poultry meat production.

Acknowledgements

Authors would like to thank the poultry producers for providing data for the study purpose, the Debre Zeit agricultural research center for the financial support and the technical staffs for their support in data collection and all responsible persons of the study area for their considerable cooperation during the data collection process.

Conflict of interest

There is no conflict of interest to declare.

References

Abdelqader, A., Gauly, M. and Wollny, C.B.A., 2007. Response of two breeds of chickens to *Ascaridia galli* infections from two geographic sources. *Vet. Parasitol.,* 145(1-2), 176–180. https://doi.org/10.1016/j.vetpar.2006.11.004.

Adamu, M., 2015. Evaluation of risk factors associated with coccidiosis in broiler farms of selected towns of Ethiopia. *African J. Basic & Appl. Sci.,* **7**(1), 34-40.

Ashenafi, H. and Eshetu, Y., 2004. Study on gastrointestinal helminthes of local chickens in central Ethiopia. *Rev. Med. Vet.,* 155, 504-507.

Ayele, G. and Rich, K.M., 2010. Poultry Value Chains and HPAI in Ethiopia, Africa/ Indonesia team working paper, *[Available from:* http://www.hpai-research.net.

Bachaya, H.A., Raza, M.A., Anjum, M.A., Khan, I.A., Aziz, A., Manzoor, Z. and Munawar, S.H., 2015. Prevalence of *Ascaridia galli* in white leghorn layers and Fayoumi-Rhode Island red crossbred flock at government poultry farm Dina, Punjab, Pakistan. *Trop. Biomed*., 32(1), 11–16.

Belete, A. and Addis, M., 2015. A survey of gastrointestinal helminthes among chickens in Bahir Dar town, Ethiopia. *Euro. J. Applied Sci*.., 7(2), 64-71.

Beyene, K., Bogale, B. and Chanie, M., 2014. Study on effects and occurrence of nematodes in local and exotic chickens in and around Bahir Dar, Northwest Ethiopia**.** *America-Euroasian J.r Scientific Res.*, 9(3), 62-66

Boere, A., Duns, H., Kidane, D., Vernooij, A. and Legesse, M., 2015. Business opportunities report poultry #3 in the series written for the "Ethiopian Netherlands business event 5–6 November., 19 pages, Rijswijk, The Netherlands".

Demeke, S., 2008. Poultry Sector Country Review: an analysis of the poultry sector in Ethiopia, Animal production and health division, emergency center for transboundary animal diseases, socio economics, production and biodiversity unit: FAO, 48 pages, Rom, Italy.

Dessie, T., and Ogle, B., 2001. Village poultry production systems in the central highlands of Ethiopia. *Trop. Anim. Health Prod*., 33(6), 521-537.

Kaufmann, F., 2011. Helminth infections in laying hens kept in alternative production systems in Germany, Prevalence, worm burden and genetic resistance. Georg-August-University Göttingen, PhD Dissertation.

Kaufmann, J., 1996. Detection of helminth eggs, larvae, oocysts, and sporocysts of protozoa in feces. 5–7 pages. In: J. Kaufmann, (ed.). Parasitic Infections of Domestic Animals (A diagnostic manual). Birkhauser Verlag, Basel, Swizerland.

Merck., 2011. Merck Veterinary manual, Overview of helminthiasis in poultry. (Nematode and cestode infections). In**:** M.K. Cynthia (Ed.)*,* MVM. 10th ed, online version, Merck Sharp & Dohme Carp, a subsidiary of Merck & Co., Inc, Whitehouse Station, NJ, USA.

Ngongeh, L.A., Chiejina, S.N. and Lawal, A.I., 2014. Prevalence of gastrointestinal helminth infections in slaughtered chickens reared in the Nsukka area of Enugu State, Nigeria. *IOSR-JAVS.,* **7**(11),51-54

Pagani, P., Wossene, A., 2008. Review of the New Features of the Ethiopian Poultry sector, Biosecurity Implications. Consultative Mission, March, FAO, pp. 1-29.

PDHA., 2009. Poultry disease hand book for Africa, Global livestock collaborative research support program, Msoffe, P.L., Cardona, C.J.:(Ed.), pp. 91.

Permin, A. and Hansen, J.W., 1998. Epidemiology, diagnosis and control of poultry parasites, Animal Health Manual, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 169.

Shane, S.M., 2005. Hand book on poultry diseases. 2nd ed, American Soybean Association (ASA), St. Louis, USA, pp. 191.

Udo, H.M.J., Asgedom, A.H. and Viets T.C., 2006. Modeling the impact of interventions in village poultry systems. *Agri. System*, 88 (2-3), 255-269.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F.W., 1996. Veterinary Parasitology. 2nd ed. Blackwell Science, Scotland, pp. 307.

Wokem, G.N. and Obiyor, E.T., 2018. Assessment of intestinal parasites of commercial layers in selected local government areas of Rivers State, Nigeria and their public health implications. *Curr. Trends in Biom. Eng & Biosci.*, 11(5), 1-5

Wossene A., 2006. Poultry Bio-security Study in Ethiopia. A consultancy report. Ministry of Agriculture and Rural Development Animal Health Department and Food and Agriculture Organization of The United Nations, April, Addis Ababa, Ethiopia, pp. 35.

Yazwinski, T., Tucker, C., Wray, E., Jones, L., Johnson, Z., Steinlage, S. and Bridges, J., 2013. A survey on the incidence and magnitude of intestinal helminthiasis in broiler breeders originating from the southeastern United States. *J. Appl. Poult. Res.,* 22, 942–947

Bovine brucellosis: Seroprevalence and its potential risk factors in smallholder dairy farms in Hawassa Town, Southern Ethiopia

*[1]Ataro Abera, [2]Yosef Deneke, [2]Tadele Tolosa**

[1]Livestock and Fishery Resource Development Department, Dawuro Zone, Tercha, SNNPRs, Ethiopia, atishabe.dvm@gmail.com, +251910197960

[2]College of Agriculture and Veterinary Medicine, School of Veterinary Medicine, Jimma University, P.O. Box: 307, Jimma, Oromia, Ethiopia, yosefdeneke@yahoo.com, +251917804529

**Corresponding author: tadeletolosa@yahoo.com, +251917804070*

Abstract

Bovine brucellosis is an infectious zoonotic disease causing significant economical loses in dairy industry. A cross-sectional study was carried out between October 2017 and July 2018 to estimate the seroprevalence and its associated risk factors in smallholder dairy farms in Hawassa town, Southern Ethiopia. A total of 370 blood samples were collected from cross-bred and local indigenous dairy cattle of above six months of age. One stage cluster sampling technique was used to get the sample of interest. Rose Bengal Plate Test (RBPT) was used as a screening, while serum samples testing positive to RBPT were subjected for complement fixation test (CFT) to confirm. Consequently, RBPT detected 18 of the 370 samples positive for brucellosis exposure. The positive sera when further retested using CFT, 10 out of the 18 RBPT positive sera were confirmed to be positive. The individual animal level prevalence of bovine brucellosis in the study area was 2.7% and the herd-level prevalence was 25.8%. Higher prevalence was observed in larger herd sizes than the small and medium herds ($p<0.05$). Likewise, parity number greater than six had more positive animals ($p<0.05$) than the corresponding group with lower parity number. Multivariable logistic regression anlysis revealed that herd sizes (OR: 9.13, 95% CI: 1.87-28.65, $p<0.05$), number of parity (OR: 11.6: 95% CI: 1.54-36.08, $p<0.05$), absence of separate parturition pen (OR: 7.9, 95% CI: 1.63-38.4, $p<0.05$) and stages of abortion (OR: 7.6, 95% CI: 1.89-31.36, $P<0.05$) were identified as the potential risk factors of bovine brucellosis. The results of this study showed that bovine brucellosis is not highly spread in dairy herds of Hawassa town. Therefore, in order to control spread of bovine brucellosis practicing better management is recommended.

Keywords: Bovine brucellosis; Dairy farms; Hawassa town; intensive; Risk factor

Introduction

Ethiopia is a resourceful country with estimated cattle population of 59.5 million (CSA, 2017). The livestock subsector has an enormous contribution to a national economy and livelihoods of many Ethiopians and still promising to rally round the economic development of the country. The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 40% of the agricultural GDP excluding the values of draught power, manure and transport of people and products (Asresie and Zemedu, 2015). It also contributes 15% of export earnings and 30% of agricultural employment (Behnke, 2010).

However, trans-boundary and zoonotic animal diseases such as bovine brucellosis constrain the livestock sector of the country and affect livelihoods via their impact on animal health, animal food production, availability and quality. Bovine brucellosis has a great impact on both animal and human health as well as tremendous socio-economic impact in developing countries where rural income relies largely on livestock breeding and dairy products (Radostits *et al*., 2007). Brucellosis is considered by Food and Agriculture Organization (FAO), World Health Organization (WHO) and World Organization for Animal health (OIE) as one of the most widespread zoonoses in the world (Schelling *et al.,* 2003). According to OIE, it is the third most important zoonotic disease in the world after rabies and anthrax. The disease affects cattle, swine, sheep, goats, camels and dogs. It may also infect other wild ruminants and marine mammals (Wadood *et al*., 2009).

The disease is primarily caused by *B. abortus* and occasionally by *B. melitensis* where cattle are kept together with infected sheep or goats and characteristically associated with abortion at first gestation ("abortion storm" in naïve heifers) and is mainly caused by biovars (mainly biotype-1) of *B. abortus* (OIE, 2009; Godfroid *et al*., 2010). Chronic infection of the mammary glands due to *B. suis* has also been reported (Lopes *et al*., 2010). Clinically bovine brucellosis is characterized by impaired fertility specifically with abortion, metritis, orchitis and epididymitis (Radostits *et al.,* 2007). The mode of transmission of the bacteria varies with the epidemiological area, the animal reservoir and the occupational exposed groups (Radostits *et al*., 2007). A precise diagnosis of

Brucella spp. infection is important for the control of the disease in animals and consequently in man.

In Ethiopia, the first cases of brucellosis reported in the 1970s. Since the first report of brucellosis the disease has been noted as one of the important livestock diseases in the country (Meyer, 1980; Tariku, 1994; Asfaw *et al*., 1998; Bekele *et al*., 2000; Alem and Solomon, 2002; Kebede *et al*., 2008; Ibrahim *et al*., 2010; Mekonnen *et al*., 2010) demonstrating brucellosis is endemic.

Brucellosis is a public health problem with adverse health implications both for animals and human beings as well as economic implications for individuals and communities. Management, animal movement, wide ranges of host, herd size, commingling of different animal species is risk factors for animal brucellosis. The possible risk factors for human brucellosis are feeding behavior, occupational exposure, contact with diseased animals or their products and discharges.

Bovine Brucellosis was studied a decade ago in the areas of Sidama zone of Southern nation nationalities and peoples regional state by Asmare *et al*. (2007) on intensive and extensive management systems. In these ten years, there is expansion of the town, increasing in population pressure and increasing dairy farms but there was no documented information on status of bovine brucellosis in study area. There are many small and medium dairy farms mushrooming which supply raw milk and milk products for the communities in Hawassa town. The demand for consumption of milk and milk products in the areas also increasing this may leads to zoonotic diseases like brucellosis. So, this study achieved the gap and provided more information on seroprevalence of bovine brucellosis and its potential risk factors that predispose the animals in the study area. The objectives of the current study were to estimate seroprevalence of bovine *Brucella* antibody circulation in small holder dairy farms of the Hawassa town and to assess the possible risk factors associated with *Brucella* antibody in the study area.

Materials and methods

Description of the Study Area

The study was conducted in Hawassa town, Southern Ethiopia between October 2017 and August 2018. Hawassa is located in the Southern Nation's Nationalities and Peoples Region on the shores of Lake Hawassa in the Great

Rift Valley and located 275 Km away from Addis Ababa in southern direction. Geographically the City lays at $6^0$55'0" latitude N and $38^0$25'0" longitudes E. The annual mean rainfall is from 800-1000 mm and annual mean temperature is between 20.1-25°C (HCAFEDD, 2017). Hawassa town is the economic and cultural hub of the region, having a total area of about 50 km^2 being divided into eight sub-cities and 32 Kebeles. It is with estimated human population of 387,087 and the main livestock populations in the town are cattle, goats and sheep and estimated to be 125,284, 39,943 and 42,190, respectively. There are 4,401 equines used for draft service. Dairy production system in Hawassa town is mostly dominated by cross breed animals which the house holds use them for production of milk to sell for the urban communities. (HTLFRDD, 2018).

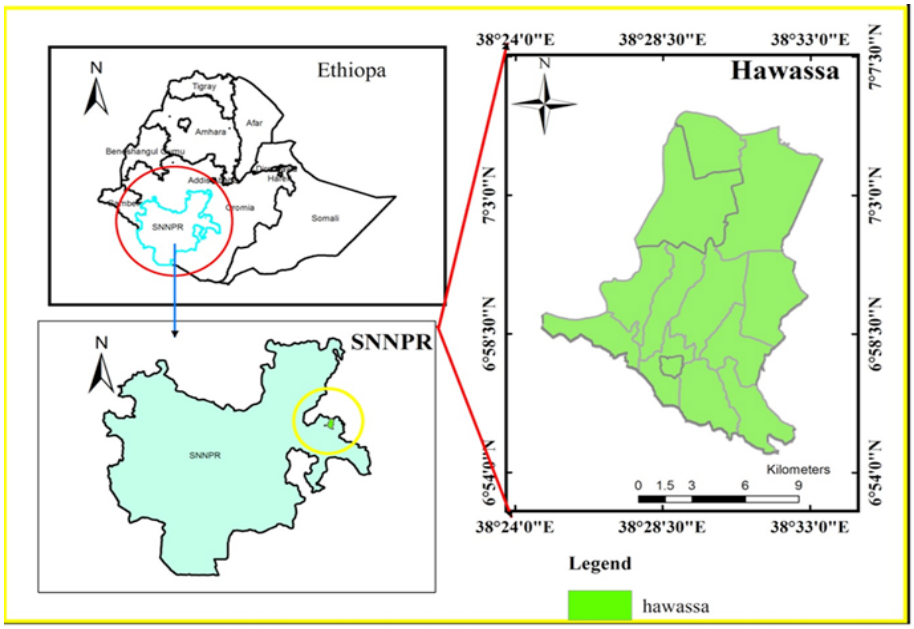


Figure 1. Map of the study area

Study animals and their management

The study consisted of dairy cattle that were managed under the intensive and semi-intensive production system. According to the criteria of Richard (1993), management systems were classified as semi-intensive husbandry system which includes all animals that both are kept indoor and outdoor while intensive system covers all animals which were kept in closed housing system and

feed concentrate as well as mixed feed. The cattle under study comprised of the cross breeds and local indigenous Zebu cattle. Animals of both sexes and different age group greater than six months were included in the study.

Dairy cows are kept under tethers feeding and some are natural grazing with supplementation of food industry byproducts. The cross-breed animals mainly the local with Holstein Frisian and Jersey are increasing in number.

Study design

A cross-sectional study design was carried out on using a pre-tested questionnaire and serological tests.

Sampling method and sample size determination

The study was included representative major dairy farms in Hawassa town. The sampling frame and sampling strategy were determined as follow:

A list of dairy farms was obtained from Hawassa town livestock and fishery resource offices and dairy owners. Farms were divided into small-scale (≤15 heads of cattle), medium-scale (≥16-29 heads of cattle) and large-scale (≥30 heads of cattle) farms depending on number of animals (Asgedom *et al*., 2016). Those cattle that housed in the same barns were grouped together and considered as one herd (Tolosa *et al*., 2008; Asgedom *et al*., 2016). A one stage cluster sampling procedure was used. The clusters were randomly selected and all animals in each cluster were sampled. There are 82 herds in the town: 52 small, 25 medium and 5 large herds. Out of these, 19 small herds (152 head of animals), 10 medium herds (158 head of animals) and 2 large herds (60 head of animals) were sampled in the study area.

The sample size for this study was calculated using 14.14% of seroprevalence of bovine brucellosis as reported by Desalegn *et al.* (2011). Therefore, to determine the sample size of dairy cattle in this area, 14.14% was used as P_{exp} and 95% confidence interval and 5% required precision (Thrusfield, 2007)

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

Where, n = required sample size,
Pexp = expected prevalence
d = desired absolute precision

Accordingly, the sample size was 186. The sample size was recalculated to get similar accuracy to that of simple random sampling. The intra-cluster correlation coefficient (ρ) of *Brucella abortus* was calculated from the results of cluster sample survey is 0.09 (Otte and Gumm, 1997). The new sample size formula (n′) was calculated by multiplying n by design effect i.e. n′= n x design effect, also Design effect=1+ρ (m-1), where m--average number of individuals sampled per cluster (m=12). Therefore, the required sample size for this study was 370 cattle above 6 months of age.

Data collection

Questionnaire survey

A total of 31 farm attendants/owners were interviewed using semi-structured questionnaire. A questionnaire survey with open and closed questions was used among the farm owners/ attendants whose farms were tested. The following data was collected on animal attributes: breed, sex, age and reproductive status, parity, stage of abortion (first trimester, second trimester and third trimester), history of abortion and retained fetal membrane and breeding systems. Based on its biological relevance, age was stratified into three categories (0.5-<3 years, ≥3-6 years and >6 years) (Asgedom *et al*., 2016). The reproductive status was also categorized (replacement heifers, pregnant cows, lactating cow, dry and bulls). Besides, information on farms such as: herd size was categorized into [small scale (≤15 heads of cattle), medium scale (≥16-29 heads of cattle) and large scale (≥30 heads of cattle)] and other managemental factors were collected. The presence of calving pens (No/Yes), waste disposal methods (placenta, aborted material and dead animal) was categorized into (burying, burning and open dump). Hygienic status of the farms was categorized as (clean and not clean) based on manure disposal, drainage and barn ventilation. Farmer's awareness about brucellosis (No/Yes) was assessed.

Blood sample collection and laboratory tests

Blood sample collection procedure

Animals were restrained by animal handlers and approximately 10 ml of blood sample was collected from the jugular vein of each animal using vacutainer tubes with 18-20-gauge hypodermic needles. Each sample from each animal

was labeled by using codes describing the specific animal and herd/farm. The samples were kept under the shade in a slant position for one hour and were centrifuged. If there was no light in the sample collection area, vacutainer tubes with serum were labeled and set tilted on a table overnight at room temperature to allow clotting. Next morning, the clotted blood in the tubes was centrifuged to obtain clear serum. The obtained serum was stored at -20^{0}C until they were tested by both Rose Bengal Plate Test and Complement Fixation Test. Corresponding to each sample, age, sex, breed of every animal, geo-reference information and other risk factors contributing to the occurrence of bovine brucellosis were collected and registered on a separate case book.

Serological tests

Rose Bengal plate test (RBPT)

It was employed as a screening test on the serum samples for the presence of *Brucella* agglutinins. The protocol of RBPT as recommended by OIE was used as screening test for the presence of *Brucella* antibody in the sampled sera. This test is generally considered to be as a sensitive test which reports to be 97.9% sensitive (Dohoo *et al*., 1986). Before performing test, antigen and sera were brought to room temperature. 30 µL of serum was taken on a glass slide by micropipette and the antigen bottle was shaken well to ensure homogenous suspension and then one drop (30 µL) of Rose Bengal antigen was added. The antigen and serum were mixed thoroughly with the spreader and then the slide was rotated for 40 min. The result was read immediately after 4 min.

Complement fixation test (CFT)

All Sera that tested positive to RBPT were further tested using CFT at the National Veterinary Institute (NVI), Debre-Zeit, Ethiopia for confirmation using standard *B. abortus* antigen S99 (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2009). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above was classified as positive and lack of fixation/complete hemolysis was considered as negative.

Data storage and analysis

Data obtained from questionnaire survey and laboratory results were recorded and stored in Microsoft® Excel for Windows 2010 and transferred to Statistical Package for the Social Sciences (SPSS) version 20. Data were coded and analyzed using descriptive and analytical statistics as appropriate. The units of analysis were individual cattle and herd. Animal level seroprevalence was computed by the number of positive animals divided by the total number of animals tested and for herd level seroprevalence the number of positive herds was divided to the total number of herds tested. Associations between outcome (brucella sero-positivity) and explanatory variables (risk factors) for all units of analysis were investigated by using binary logistic regression model. The strength of the association between outcome (brucella sero-positivity) and explanatory variables was assessed using the adjusted odds ratios (OR). Multivariable logistic regression procedures were used to model the effects of potential risk factors on outcome variables. The backward elimination procedure was used to eliminate the factors that were not significant at $p<0.05$ in the overall model. Model fit was observed using the Hosmer-Lemeshow test. Subsequently, the predictive ability of the model was validated using the receiver operating characteristic (ROC) curve. In the analysis, a covariate was considered confounder and included in the model if its inclusion altered the OR of the estimated risk by more than 20% (Dohoo *et al*., 2009).

Results

Questionnaire survey

A total of 31 volunteer farm attendants and owners in the farms were interviewed to assess the awareness about brucellosis. Majority of cattle attendants and owners (54.8%) have no knowledge about brucellosis (Table 2). The vast majority of the interviewees (93.5%) were male. Forty-five percent of the respondents in the study area can write and read (Table 1). Fifty five percent of the participants have poor knowledge of the disease. However, the level of awareness was insignificantly lower ($p>0.05$) in intensive farms.

Table 1. Socio-demographic Characteristics of Respondents

Variables	Categories	Frequency	Percentage (%)
Sex of household	Male	29	93.5
	Female	2	6.5
Age of household	b/n 25-35	10	32.3
	b/n 36-46	12	38.7
	≥47	9	29
Educational status of households	Illiterate	10	32.3
	write and read	14	45.2
	Primary	5	16.1
	high school	2	6.5
	higher education	0	0.0
Total		31	100.00

Almost all of respondents practice intensive husbandry system 28 (90.3%) and only 3(9.7%) from the semi-intensive management systems. In intensive farms, artificial insemination (AI) is used for breeding in herds with cross breeds while most herds in semi-intensive farm use bull for breeding. Among intensive farms, 61.3% of farms were regarded as clean however, 38.7% of intensive farms and all of the semi-intensive farm management systems had poor hygienic practices including poor waste disposal, drainage and poor barn ventilation observed. Around 20% of respondents did not dispose waste products (aborted material or afterbirth) properly (Table 2). Generally, the frequency distribution of breed, parity, age group, reproductive status, retained fetal membrane and hygienic practice on farm were summarized in the following table (Table 2 and 3).

Table 2. Respondents' response on management of dairy farm practices

Parameters	Categories	Frequency	Percentage (%)
Hygienic practice on farm	Not clean	12	38.70
	Clean	19	61.30
Level of awareness about brucellosis	No	17	54.80
	Yes	14	45.16
Animal introduction	No	20	64.50
	Yes	11	35.50
Separate parturition pen	No	14	45.16
	Yes	17	54.80
Management system	Intensive	28	90.30
	Semi-intensive	3	9.70
Breeding system	Natural mating	8	25.80
	AI	17	54.80
	Both	6	19.40
Disposal of aborted fetus/fetal membrane	Burning	14	45.16
	Burying	8	25.81
	Both	3	9.68
	open dump	6	19.35
Total		31	100.00

Out of 370 dairy animals, 105 (28.38%) were local breed whereas 265 (71.62%) were cross-breeds of indigenous zebu and Holstein Friesian. In addition, the herd characteristics of the studied animals were 107 (28.92%) milking cows, 21 (5.68%) pregnant cows, 126 (34.05%) non milking cows, 68 (18.38%) replacement heifers and 48 (12.97%) bulls. From the total studied animals, there was a history of retained fetal membrane in 29 (9.00%) and 25 (7.76%) were with a history of abortion (Table 3).

Table 3. Frequency distribution of individual animal variables and percentage

Variables	Categories	Frequency	Percentage (%)
Breed	Local	105	28.38
	Cross	265	71.62
Reproductive status	Heifer	68	18.38
	Milking cow	107	28.92
	Non-milking cow	126	34.05
	Bull	48	12.97
	Pregnant cow	21	5.68
History of reproductive problems	No	268	72.43
	Abortion	25	7.76
	RFM	29	9.00
Sex	Male	48	12.97
	Female	322	87.03
Body condition score	Poor	160	43.24
	Medium	136	36.76
	Good	74	20.00
Age	0.5-<3	63	17.03
	≥3-6	129	34.86
	>6	178	48.11
Total		370	100.00

Overall seroprevalence

Individual animal level seroprevalence of bovine brucellosis

Out of 370 sera samples collected and screened by RBPT, 18 (4.86%) (95% CI: 2.67% -7.10%) were seropositive for brucella antibody. Among 18 brucella positive reactors, 10 (2.70%) (95% CI: 1.05% - 4.36%) of them were confirmed to be seropositive by CFT test. Animals with the age between 0.5-<3 years, replacement heifers, animals which abort at the stage of first trimester and semi-intensive management system tested were negative in both RBPT and CFT.

Table 4. Animal level risk factors and brucella seropositivity in Hawassa town

Risk factors	Categories	No of tested	No of positive	Prevalence (%)	95% CI	P-value
Body condition score	Poor	160	6	3.75	0.81-6.69	0.38
	Medium	136	2	1.47	0.55-3.49	
	Good	74	2	2.70	0.99-6.40	
Sex	Male	48	1	2.10	0.15-16.15	0.17
	Female	322	9	2.80	0.30-0.92	
Parity	No	75	1	1.33	1.26-3.93	0.04
	1-3	129	1	0.78	0.74-2.29	
	4-6	67	2	2.99	1.09-7.06	
	>6	51	5	9.38	1.64-17.97	
Stage of abortion	Never	75	4	5.33	0.53-3.4	0.03
	1st trimester	129	0	0.00	0.00-2.89	
	2nd trimester	67	3	4.48	0.47-9.43	
	3rd trimester	51	3	5.88	0.58-12.34	
Breed	Local	105	4	3.8	0.15-7.47	0.92
	Cross	265	6	2.26	0.47-4.1	
Reproductive status	Heifer	68	0	0.00	0.00-0.06	0.48
	Milking cow	107	4	3.74	0.14-7.33	
	Non-milking cow	126	4	3.17	0.11-6.24	
	Bull	48	1	2.1	1.96-6.12	
	Pregnant cow	21	1	4.76	4.35-13.87	

CI: Confidence interval

Table 5. Herd level risk factors and *brucella* seropositivity in Hawassa town

Risk factors	Categories	No of tested	No of positive	Prevalence (%)	95% CI	P-value
Herd size	≤15	19	1	5.26	4.78-15.30	0.04
	16-29	10	4	40.00	9.64-70.36	
	≥30	2	2	100.00	100-100	
Hygienic practice on farm	Not clean	12	2	16.67	4.42-37.75	0.26
	Clean	19	6	31.6	10.68-52.5	
Animal introduction	No	20	6	30.0	9.92-50.1	0.83
	Yes	11	2	18.18	4.61-40.97	
Separate parturition pen	No	14	6	42.86	16.93-68.8	0.04
	Yes	17	2	11.76	3.6-27.1	
Management system	Intensive	28	8	28.57	11.84-45.3	0.82
	Semi-intensive	3	0	0.00	0.00-0.00	
Breeding system	Natural mating	8	4	50.0	15.35-84.7	0.54
	AI	17	3	17.7	0.47-35.77	
	Both	6	1	16.67	13.15-46.5	

CI: Confidence interval

Herd-level seroprevalence of bovine brucellosis

Out of 31 herds included in the study, 8 herds were sero-positive with at least one seropositive animal in the herd (8/31) (25.8%) (95% CI: 10.4%-41.2%). The range of herd level prevalence was within 5.56% up to 8.33% which at least one seropositive animal was observed based on CFT. In this study herds with larger herd size (greater 30 animals) has significantly higher prevalence than herds with small herd size ($p<0.05$). The value of OR indicated that herds in the herd size of 16-29 animals were about 9.13 times more likely to be seropositive than herds in the ≤15 heads of animals. However, management systems ($p>0.05$), Body condition score ($p>0.05$ Sex ($p>0.05$) and Breed ($p>0.05$) were not associated with seropositivity of brucellosis (Table 4 and 5).

Table 6. Univariable logistic regression analysis of factors associated with *Brucella* seropositivity

Variables	Categories	No of tested animals	No of CFT positive (%)	OR (95% CI)	P-value
Sex	M (Ref)	48	1(2.1)		
	F	322	9(2.8)	4.1 (0.6-8.26)	0.17
Herd size	≤15 (Ref)	152	1(0.66)		
	≥16-29	158	5(3.16)	2.56(0.01-4.8)	0.05
	≥30	60	4(6.67)	3.4(0.2-4.08)	0.04
Parity	No (Ref)	75	1(1.33)		
	1-3	129	1(0.78)	0.87(0.01-2.96)	0.26
	4-6	67	2(2.99)	4.6(1.32-6.52)	0.09
	>6	51	6(9.38)	5.42(2.04-7.62)	0.04
Stage of abortion	Never	75	4(5.33)		
	1st trimester	129	0(0.00)	0.8 (0.03-0.98)	0.82
	2nd trimester	67	3(4.48)	7.4 (0.49-42.8)	0.03
	3rd trimester	51	3(5.9)	3.54(0.98-7.23)	0.05
Breed	Local	105	4(3.8)	0.122(0.01-1.67)	0.92
	Cross (Ref)	265	6(2.26)		
Management system	Intensive	334	10(2.99)	0.57(0.06-0.87)	0.82
	Semi-intensive (Ref)	36	0 (0.00)		
Observed abortion/RFM	No	268	2 (0.75)	0.4 (0.02-14.9)	0.29
	Yes (Ref)	54	7 (12.96)		
Separate parturition pen	No	167	6(3.6)	4.72(0.98-15.24)	0.04
	Yes (Ref)	203	4(1.97)		

OR: Odd ratio; CI: Confidence interval; Ref: Reference

Potential risk factors

Variables with a $p<0.25$ in the univariable analysis were included in the final multivariable logistic model. Two variables, age and parity that showed collinearity with each other, so age was removed from the model and parity stayed in the model. The rest variables; parity, herd size, separate parturition pen, stage of abortion was offered to the model. Further selection of variables in the final model was based on stepwise backward elimination procedure. A Hosmer-Lemeshow goodness-of–fit value ($p=0.63$), indicated that the model

was fit the data. The area under the ROC curve was 0.54, indicating that the model had good predictive ability.

The sero-prevalence was significantly higher in the greater than six parity numbers of animals (9.38%) than the parity numbers between 4-6 ones (2.99%), consequently the seroprevalence increases as the parity of the animals increase. This study revealed that there is association between parity and seropositivity of bovine brucellosis with ($p< 0.05$).

Higher prevalence was observed in larger herd size (6.67%); old animals were affected more than young animal and no brucella reactors were observed in young animals in this finding. Seroprevalence of 12.0% was observed in animals with previous history of abortion.

The final multivariable logistic regression model showed that parity, absence of separate parturition pen, herd size and stage of abortion were independently associated with seroprevalence of bovine brucellosis. The multivariable logistic regression model revealed that herd size (OR: 9.13; 95% CI: 1.87-28.65, $p<0.05$), stage of abortion (OR: 7.6; 95% CI: 1.89-31.36, $p<0.05$), separate parturition pen (OR: 7.9; 95% CI: 1.63-38.4, $p<0.05$) and parity (OR: 11.6; 95% CI: 1.54-36.08, $p<0.05$) were potential risk factors for cattle seropositivity to circulating brucella antibodies (Table 7).

Table 7. Multivariable logistic regression analysis of potential risk factors with Brucella seropositivity

Variables	Categories	No of tested animals	No of CFT positive (%)	OR (95% CI)	P-value
Herd size	≤15 (Ref)	152	1(0.66)		
	16-29	158	5(3.1)	2.45(1.07-3.98)	0.034
	≥30	60	4(6.67)	9.13(1.87-28.65)	0.001
Separate parturition pen	No	167	6(3.6)	7.9(1.63-38.4)	0.021
	Yes (Ref)	203	4(1.97)		
Stage of abortion	Never (Ref)	75	4(5.33)		
	1st trimester	129	0(0.00)	1.8(1.2-2.14)	0.043
	2nd trimester	67	3(4.48)	5.5(1.07-18.2)	0.004
	3rd trimester	51	3(5.9)	7.6(1.89-31.36)	0.001
Parity	No (Ref)	75	1(1.33)		
	1-3	129	1(0.78)	2.45(0.35-4.03)	0.128
	4-6	67	2(2.99)	6.12(1.14-11.08)	0.041
	>6 (Ref)	51	6(9.38)	11.6(1.54-36.08)	0.001

OR: Odd ratio; CI: Confidence interval; Ref: Reference

Discussion

Awareness about brucellosis among farmers is crucial in controlling disease transmission. In this study, farm attendants/herd owners were interviewed to assess their awareness levels about brucellosis using semi-structured questionnaire. All farmers interviewed had no similar awareness about brucellosis and farmers which were aware about brucellosis were significantly lower in both farming systems. It also observed that poor hygienic practices and uncontrolled animal movements were practiced in semi-intensive husbandry systems. These could pose high risks of transmitting the disease within and in between the herds. This is in agreement with previous studies in intensive farming in Ethiopia by Tesfaye *et al*. (2011); Asgedom *et al*. (2016); Elemo and Geresu. (2018) and Waktole *et al*. (2018).

This low level of educational status may lead to reduced production of dairy farms because of low use of dairy innovations such as cultivation of improved forages, breeding techniques and use of modern dairy farming in the study area. This study revealed that the occurrence of abortion and retained fetal membrane was due to lack of knowledge on breeding methods and disease transmission, shortage of feed and lack of awareness on isolation of aborted animal from healthy animals could possibly be associated with the high prevalence rate of reproductive problems.

Similar prevalence to the current study of bovine brucellosis based on RBT and CFT has been reported from the highland areas of Ethiopia among cattle in intensive production systems (Asmare *et al*., 2004; Kebede *et al*., 2008; Jergefa *et al*., 2009). However; in certain parts of the Ethiopia some authors observed lower prevalence in indigenous cattle under intensive production systems by using the same diagnostic tests (Tolosa *et al*., 2010; Alemu *et al.,* 2014; Geresu *et al*., 2015; Asgedom *et al*., 2016). This variation could be due to differences in cattle management systems, husbandry practices.

The current finding was consistent with earlier findings of Asmare (2004) who reported 2.5% prevalence in Sidama zone dairy farms by using the same tests and management practices; Waktole *et al*. (2018) who reported a prevalence of 3% in selected dairy farms of Bishoftu town, Oromia region; Asmare *et al*. (2007) documented a seroprevalence of 2.46% in sidama zone and Tesfaye *et al*. (2017) who observed 2.08% seroprevalence in and around Kombolcha, Amhara regional state.

However, in Ethiopia a lower seroprevalence which contradict the current study were documented in previous findings of Bashitu *et al*. (2015) who observed a seroprevalence of 0.2% which conducted on 415 animals by using RBPT and CFT tests in Debrebirhan and Ambo towns and Sarba *et al*. (2016) who reported an overall seroprevalence of 0.49% on 816 animals using the same tests in selected towns of West Shewa, Ethiopia; Geresu *et al*. (2016) who observed 1.4% seropositivity of brucella on 570 animals using RBPT, CFT CT and i-ELISA tests in dairy cows in Asella and Bishoftu towns, Oromia regional state, Ethiopia and Tesfaye *et al*. (2011) who reported 1.5% prevalence in Addis Abeba dairy farms by using the same tests.

In the contrary, so far higher seroprevalence findings are reported by Elemo and Geresu (2018) who observed prevalence of 4.95% on 768 animals using RBPT and CFT tests in smallholder farms of Agarfa and Berbere districts of Bale Zone, South Eastern Ethiopia; Kebede *et al*. (2008) reported an overall seroprevalence of 11.0% on 1136 cattle using RBPT and CFT tests in Wuchale-Jida district; Alehegn *et al*. (2016) who reported 4.9% seroprevalence in and around Gondar Town, North West Gondar; Desalegn *et al*. (2011) who observed 14.14% of prevalence using the same tests in Assella Government Dairy Farm of Oromia Regional State, Ethiopia and Hailemelekot *et al*. (2007) who observed 4.6% of prevalence using the same tests in selected sites of Ethiopia.

The difference in seroprevalence might be due to the difference in management systems, age of the animals, sample sizes, parity of the animals, herd sizes and sex among dairy farms. It has been reported that susceptibility of cattle to *B. abortus* infection is influenced by age of an individual animal. Thus, sexually matured and pregnant cattle are more susceptible to infection with *Brucella* organisms than sexually immature animals of either sex. On the other hand, younger animals tend to be more resistant to infection and frequently clear infections, although latent infection may occur. This may be due to the fact that sex hormones and erythritol, which stimulates the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits *et al*., 2007)

This study also estimated that there is association between parity and seropositivity of bovine brucellosis and hence, parity was one of the potential risk factors in the study area. This is probably due to increased contact with fetal materials and vaginal discharge from infected cows there by increasing the chance of being infected by *brucella* species. This finding was in agreement

with the findings of other authors (Desalegn *et al*., 2011; Elemo and Geresu, 2018).

Even though age was not significantly associated with brucella seropositivity, a seroprevalence of 3.37 was found among age group of >6 years whereas no brucella seropositivity was observed in in the younger age group (6 months up to less than three years) of dairy cattle in the study area. Several earlier reports have indicated that the higher seroprevalence of brucellosis in adult age group of cattle which in contour with the findings of Magona *et al*. (2009) similar to the findings of this study. This report was in line with literatures which supports younger animals tend to be more resistant to infection and frequently clear infections. Sexually mature animals are more susceptible to Brucella infection than sexually immature animals, which are due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of Brucella organism, tend to increase in concentration with age and sexual maturity (Walker, 1999; Radostits *et al*., 2007).

There was statistically significant association between ($p<0.05$) stage of abortion and seropositivity of brucella in the present study. This could be explained by the prevalence of higher seroprevalence in cows in the last trimester may due to the preferential localization of brucella in the uterus in which allantoic fluid factors such as erythritol could stimulate the growth of brucella and elevate in the placenta and fetal fluid from about the fifth months of gestation (Coetzer and Tustin, 2004; Radostits *et al*., 2007).

In the present study, statistically significant variation has been observed in seroprevalence of brucellosis between different herd sizes; larger herd sizes were nine times more likely to be seropositive. Herd size has previously been reported as an important determinant for transmission of *Brucella* organism between susceptible and infected animals (Omer *et al*., 2000) and thus; larger herds were more likely to have at least one positive animal than smaller herds (Al-Majali *et al*., 2008). In larger herd sizes, the disease spreads by several modes of transfer, especially through contact with infected discharges from dam and its fetus (Radostits *et al.,* 2007). Thus, brucellosis should never be viewed as the disease of individual animals, but should be considered in the context of herd and also the animal population in the region. However, in contrary to this Kebede *et al*. (2008), who reported that the risk of seropositivity was independent of herd size in Wuchale Jida district of East Wollega zone of Ethiopia. The observed variation of the reports among different region of

Ethiopia and other countries could be attributed to various factors including agro-ecology, management system. In the current finding the absence of separate parturition pen in the study area was significantly associated with brucella seropositivity. This is due to hygienic problems in dairy farms in which the sanitary systems of farm are not clean. This predisposes the animals to the disease.

In epidemiological studies, the use of two tests applied serially is recommended to maximize the accuracy of test results. A combination of rose Bengal and complement fixation tests is the most widely used serial testing scheme. Rose Bengal test is highly sensitive test and could easily apply in field conditions whereas, complement fixation test is highly specific usually used as a confirmatory test method (Samui *et al*., 2007). The combination of these tests in this study could therefore maximize the accuracy of the findings. The false positive results in the RBT could be due to cross reactions with other bacteria such as *Yersinia enterocolitica*, *E. coli*, *Salmonella spp*. and *Pastuerella spp*.

Conclusion

In the current study, the seroprevalence recorded revealed that brucella antibody circulation is an established disease in dairy farms of Hawassa town. The current finding revealed that large herd sizes, absence of separate parturition pen, animals with the highest number of parity and animals which aborted at last trimester were identified as potential risk factors of brucella seropositivity. Even though age was not significantly associated with brucellosis, adult animals were highly predisposed than young animals and almost all of the positive reactors were female animals. From questionnaire survey, poor hygienic practices like improper disposal of aborted fetuses and fetal membranes were identified as potential risk factors which could create favorable condition for the entry and establishment of bovine brucellosis in the dairy farms. In conclusion, the prevailing *Brucella* seropositivity in the dairy farms indicates the disease has a major impact on human health, besides causing significant economic losses in dairy industry. Hence, using calving pens, improving hygiene, and awareness creation to farm attendants/owners are recommended to control further spread of the disease.

Acknowledgements

Authors would like to acknowledge Jimma University, College of Agriculture and Veterinary Medicine academic and support staff members, for their positive cooperation during the study.

References

Alehegn E., Tesfaye S. and Chane M., 2016. Seroprevalence of Bovine Brucellosis and its Risk Factors in Cattle in and around Gondar Town, North West Gondar, Ethiopia. *J Dairy Vet. Anim. Res.,* 4 (4), 166.

Alem W. and Solomon G., 2002. A retrospective sero-epidemiology study of Bovine Brucellosis in different Production Systems in Ethiopia. In Proceeding of 16th Annual Conference, Addis Ababa, Ethiopia. Pp. 53-57.

Alemu F., Admasu P., Feyera T. and Niguse A., 2014. Seroprevalence of Bovine Brucellosis in Eastern Showa, Ethiopia. *Acad. J. Anim. Dis.,* 3 (3), 27-32.

Al-Majali, A. M., Abdelsalam, Q. T., Mustafa, M. A. and Mohammed, M. A., 2008. Seroprevalence and risk factors for bovine brucellosis in Jordan. *J. Vet. Sci.*, 10, 61-65.

Asfaw, Y., B. Molla, B., Zessin H. K. and Tegene A., 1998. The Epidemiology of Bovine Brucellosis in Intra and Peri-urban Dairy Production Systems in and around Addis Ababa. *Bull. Anim. Hlth. Prod. Afr.*, 46, 217-224

Asgedom, H., Damena, D., and Duguma, R., 2016. Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia. *Springer Plus*; 5: 851.

Asmare K. 2004. Epidemiology of Brucellosis in Cattle and its Sero Prevalence in Animal Health Professionals in Sidama Zone, Southern Ethiopia. MSc Thesis, Addis Ababa University, College of Veterianry Medicine. Debre Zeit, Ethiopia.

Asmare, K., Shiv P, Yilkal A, Esayas, G, Gelagaye A, Aschalew Z., 2007. Seroprevalence of brucellosis in cattle and high-risk professionals in Sidama Zone, Southern Ethiopia. *Ethiop. Vet. J.*, 11, 69-84.

Asresie, A., and Zemedu, L., 2015. Contribution of livestock sector in Ethiopian economy: A Review. *Advanced Life Sci. Tech.,* 29, 79-90.

Bashitu, L., Afera B, Tuli G, Aklilu F., 2015. Sero-prevalence Study of Bovine Brucellosis and its Associated Risk Factors in Debrebirhan and Ambo Towns. *J. Dairy Ve.t Anim. Res.,* 3, 131.

Behnke, R., 2010. The contribution of livestock to the economies of IGAD Member States: Study findings, application of the methodology in Ethiopia and recommen-

dations for further Work. IGAD LPI Working Paper 02-10. Great Wolford, UK: Odessa Centre, IGAD Livestock Policy Initiative.

Bekele, A., Molla, B., Asfaw, Y., Yigezu, L., 2000. Bovine brucellosis in ranches and farms in Southeastern Ethiopia. *Bull. Anim. Hlth. Prod., Afr*., 48, 13-17.

Coetzer, J.W., and Tustin, R.C., 2004. Infectious diseases of livestock. 3rd ed. South Africa, Oxford University press, Pp: 34-39.

CSA, 2017. Federal Democratic Republic of Ethiopia Central Statistical Agency. Agricultural sample survey 2016/17. Report on livestock and livestock characteristics. Statistical Bulletin, Pp. 1-188.

Desalegn, F., Berhe, T. and Gangwar S. K.., 2011. Seroprevalence Study of Bovine Brucellosis in Assella Government Dairy Farm of Oromia Regional State, Ethiopia. *I.J.S.N.*, 2(3), 692-697.

Dohoo, I., Wright, P., Ruckerbauer, G., Samagh, B., Robertson, F., *et al*., 1986. A comparison of five serological tests for bovine brucellosis. *Can. J. Vet. Res.*, 50, 485-493.

Dohoo, I., Martin, W., Stryhn, H., 2009. Veterinary epidemiologic research, 2nd ed. AVC, Charlottetown, Prince Edward Island, Pp. 239-249.

Elemo, K. K., and Geresu. M. A., 2018. Bovine brucellosis: Seroprevalence and its associated risk factors in cattle from smallholder farms in Agarfa and Berbere districts of Bale Zone, South Eastern Ethiopia. *J. Anim. Plant Sci.*, 28 (2).

Geresu, M. A., Ameni, G., Kassa, T., Tuli, G., Arenas, A., and Kassa, G.M., 2016. Seropositivity and risk factors for brucella in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. *Afr. J. Microbiol. Res.*, 10 (7), 203-213.

Godfroid, J., Nielsen, K. and Saegerman, C., 2010. Diagnosis of brucellosis in livestock and wildlife. *Croat. Med. J.*, 51, 296-305.

Hailemelekot M, Kassa T, Tefera M, Belihu K, Asfaw Y, Ali A., 2007. Seroprevalence of brucellosis in cattle and occupationally related humans in selected sites of Ethiopia. Ethiop. Vet. J., 11, 85-100.

HCAFEDD, 2017. Hawassa City Administration Finance and Economic Development Department. Socio-economic and Geo-spatial data analysis and dissemination core work process. Socio-economic profile. (unpublished).

HTLFRDD, 2018. Hawassa town livestock and fishery resource development department. (unpublished).

Ibrahim, N., Belihu, K., Lobago, F. and Bekana, M., 2010. Sero-prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. *Trop. Anim. Health. Prod.,* 42, 34-40.

Jergefa, T., Kelay, B., Bekana, M., Teshale, S., Gustafson H. and Kindahl, H., 2009.Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. *Rev. Sci. Tech.*, 28 (3), 933–943.

Kebede, T., Ejeta, G. and Ameni, G., 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). *Revue Méd Vét.*, 159, 3-9.

Lopes, L. B., Nicolino, R. and Haddad, J. P. A., 2010. Brucellosis-risk factors and prevalence: A review. *Open. Vet. Sci. J.*, 4, 72-84.

Magona, W. J., Walubengo, J., Galiwango, T. and Etoori, A., 2009. Seroprevalence and potential risk factors of bovine brucellosis in zero grazing and pastoral dairy systems in Uganda. *Trop. Anim. Hlth. Prod.,* 41:1765-1771.

Mekonnen, H., Kalayou, S., and Kyule, M., 2010. Serological survey of bovine brucellosis in Barka and Arado breeds (*Bos indicus*) of western Tigray, Ethiopia. *Prev. Vet. Med.*, 94 (1-2), 28-35.

Meyer, M. E. (1980). Report on Veterinary Activities, Institute of Agricultural Organization Research, Ethiopia. Food and Agriculture organization of the United Nations, Rome, Italy. Pp: 24.

Office International des Epizooties (OIE), 2009. Bovine brucellosis in terrestrial manual; http://www.oie.int/.

Omer, M. K., Skejerve, E., Holstad, G., Woldehiwet, Z., and Macmillan, A. P., 2000. Prevalence to Brucella species in cattle, sheep, goat, horses and camels in the state of Eritrea; influence of husbandry system. *Epidemiol. Infect.*, 125, 447-453.

Otte, M. G., and Gumm, I. D., 1997. Intra-cluster correlation coefficients of 20 infectious calculated from the results of cluster sample surveys. *Prev. Vet. Med.,* 31, 147-150.

Radostits, O. M., Gay, C. C., Hinchcliff, K.W., Constable, P. D., 2007. Veterinary Medicine. A text book of Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th Ed. W. B.,Saunders, London, Pp. 963-985.

Richard, W., 1993. Dairying. Tropical Agriculturalist, 1st Ed. Macmillan Press, London. Pp. 43-48.

Samui, K. L, Oloya, J, Munyeme, M, Skjerve, E., 2007. Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. *Prev. Vet. Med.*, 80, 306-317.

Sarba, E. J., Getaneh A. M., Borena B. M., Ambecha H. A., Berecha M. S., Eteya W. T., Tola G. K. 2016. Seroprevalence and associated risk factors of brucellosis in dairy cattle in selected towns of West Shewa, Ethiopia. *Bull. Anim. Hlth. Prod. Afr.*, 64, 387-395.

Schelling, E., Diguimbaye, C., Daoud, S., *et al*., 2003. Brucellosis and Q fever seroprevalence of nomadic pastoralists and their livestock in Chad. *Prev. Vet. Med.*, 61, 279-293.

Tariku, S., 1994. The impact of brucellosis on productivity in improved dairy herd of Chaffa State Farm, Ethiopia. Berlin, Frei universitate, Fachburg Veterinaemedizin.

Tesfaye, G., Tsegaye, W., Chanie, M. and Abinet, F., 2011: Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms. Trop. Anim. Hlth. Prod., 43, 1001-1005.

Tesfaye, G., Wondimu, A., Asebe, G., Regasa, F., Mamo, G., 2017. Sero-prevalence of Bovine Brucellosis in and Around Kombolcha, Amhara Regional State, Ethiopia. Mycobacter. Di., 7(2), 242

Thrusfield, M. 2007. Sample size determination. In: Veterinary Epidemiology. 3rd ed., UK: Blackwell Science Ltd, Pp.185-189.

Tolosa, T., Regassa, F., and Belihu, K., 2008. Seroprevalence study of bovine brucellosis in extensive management system in selected sites of Jimma zone, western Ethiopia. Bull. Anim. Hlth. Prod. Afr., 56, 25–37.

Tolosa, T., Bezabih, D., and Regassa, F., 2010. Study on seroprevalence of bovine brucellosis, abortion and associated risk factor. Bull. Anim. Hlth. Prod. Afr., 58, 236-247.

Wadood, F., Ahmad, M., Khan, A., Gul, S. T., and Rehman, N., 2009. Seroprevalence of brucellosis in horses in and around Faisalabad. Pak. Vet. J., 29, 196-198.

Waktole, H., Geneti, E., Ahmed, W.A., Mammo, G., and Abunna, F., 2018. Seroprevalence and risk factors of bovine brucellosis in selected dairy farms in Bishoftu, Oromia, Ethiopia. Intl. J. Microbiol. Res., 9 (2), 45-53.

Walker, R. L., 1999. Brucella. In: Hirsh D. C, Zee Y. C (eds.) Veterinary Microbiology Malden, USA, Blackwell Science, Pp. 196-203.

Prevalence of bovine trypanosomosis and assessment of knowledge and practices of livestock owners in the control of Trypanosomosis in Assosa District of Benishangul Gumuz Regional State, Ethiopia

Fantahun Miteku[1], Wagari Tafese[1] and Tsegaw Fentie[2]*

[1]Veterinary Drugs and Animal Feed Administration and Control Authority, Addis Ababa

[2] College of Veterinary Medicine and Animal Science, University of Gondar, Gondar, Ethiopia. Corresponding author; Fantahun Miteku email: jarrafantahun@gmail.com

Abstract

A cross-sectional study was conducted from December 2013 to February 2014 to estimate the prevalence of bovine trypanosomosis and identify species of trypanosomes infecting cattle. Besides, attempt was made to assess the knowledge and practice of the livestock owners in Assosa district of Benishangul Gumuz Regional State, Ethiopia. A total of 310 blood samples were collected from randomly selected cattle. Packed cell volume (PCV) was measured and samples were examined for the presence of trypanosomes using the buffy coat technique. In this study bovine trypanosomes were detected in 22.9% of the blood samples. The most common trypanosome species identified were *T. congolense* 13.2% (41/310) followed by *T. brucei* 3.2% (10/310), *T. vivax* 2.9% (9/310), mixed infections of *T. vivax* and *T. congolense* 1.6% (5/310), *T. congolense* and *T. brucei* 1.29% (4/310) and *T. brucei* and *T. vivax* 0.64 (2/310). There were no statistically significant prevalence differences ($p > 0.05$) between sexes, ages category and management systems. However, prevalence difference was noted between poor and good body condition categories ($p < 0.05$). Animals with poor body condition were found 2.11 times more likely of getting trypanosome infection (OR=2.11, 95% CI, p=0.027) than animals in good body condition. The mean PCV values recorded were 23.3% in parasitaemic and 25.1% in aparasitaemic animals with a statistically significant difference ($p<0.05$). A questionnaire survey was conducted for the assessment of knowledge of farmers on control and prevention of bovine trypanosomosis provided important information on farmer's knowledge of bovine trypanosomosis and practices in control and prevention measures. In this study few livestock owners know the vector (tsetse) association with the disease. In conclusion, this study confirmed that trypanosomes are still a threat to cattle in the study area. Therefore, it is

recommended that an integrated prevention and control program have to be designed and implemented to minimize their impacts on livestock production.

Keywords: Assosa; Cattle; Knowledge assessment; Trypanosomosis; Practices, Prevalence

Introduction

Livestock is a significant contributor to economic and social development in Ethiopia at the household and national level. On a national level, livestock contributes a significant amount to export earnings in the formal market (10% of all formal export earnings or $150m annually) and the informal market (likely $300m+ annually). Livestock accounts for 15-17% of total GDP and 35-49% of agricultural GDP. Livestock directly contributes to the livelihoods of more than 70% of Ethiopians. Ethiopia has the largest livestock herd in sub-Saharan Africa, with an estimated cattle population of 49 million, sheep population of 25 million and goat population of nearly 22 million (EATA, 2014).

Tsetse transmitted animal trypanosomosis still remains as one of the largest causes of livestock production losses in Ethiopia. About 10 to 15% of the land believed to be suitable for livestock production is affected by one or two species of the tsetse flies. While tsetse-borne trypanosomosis is excluding agriculturally suitable land of the country; 14 million head of cattle are at the risk of contracting trypanosomosis at any one time (Desta *et al*, 2013).

According to Abebe (2005), trypanosomosis is prevalent in two main regions of Ethiopia that is, the Northwest and the Southwest regions. Six species of trypanosomes are recorded in Ethiopia and the most important trypanosomes, in terms of economic loss in domestic livestock are the tsetse transmitted species: *T. congolense, T. vivax* and T. *brucei.* For the closely related *T. brucei* subspecies, *T. b. rhodensiense*, which causes human sleeping sickness, cattle can be a reservoir host. The other trypanosome species of economic importance are *T. evansi* of camels and *T. equiperdum* of horses (Abebe, 2005). Recent findings and field observations have indicated that the common trypanosomosis control tools; i.e., trypanocurative and trypanoprophylactic drugs have become ineffective in many areas due to development of drug resistance by the parasite. Moreover, toxicity of the drugs and exhibition of antigenic variation which hampers vaccine production are the limitations facing the modern vet-

erinary medicine (Uilenberg, 1998). Though trypanosomosis is a problem for cattle production in the study area, information is limited to the magnitude of the disease and farmers' perception on the presence, impact and management of trypanosomosis in cattle, and their desire for establishment of intervention programs against the disease Thus, the objectives of this study were to estimate the prevalence of bovine trypanosomosis and associated risk factors in the district; and to assess the knowledge and practices of livestock owners towards the control of bovine trypanosomosis.

Materials and methods

Description of study area and population

The study was conducted from December 2013 to February 2014 in Assosa Zone of Benishangul Gumuz Regional State (Figure 1). The area stretches over 2313 km^2 in a major tsetse and tsetse born trypanosomosis belt characterized by lowland plane with altitude range of 580-1544 meter above sea level. Assosa is located between 8°30"and 40°27" N and 34°21" and 39°1" E. According to National Meteorological Service, the average annual rainfall is 1316 mm with uni-modal type of rainfall that occurs between April and October. Its mean annual temperature ranges between 16.75°C and 27.9°C. Assosa zone has 35.6% of the livestock population of the region constituting 81,939 cattle, 73,181 goats 10,231 sheep, 14,089 donkeys, 40, 3153 poultry, 29 horses and 59,695 beehives (CSA, 2005). The study was carried out on indigenous zebu cattle of all age groups of both sexes in five selected kebeles in and around Assosa town which are managed mostly under extensive and semi-intensive production systems.



Figure 1: Map of study area, Assosa district in Benishangul Gumuz Regional State
Source: From Wikipedia, the free encyclopedia

Study design

A cross-sectional study was conducted from December 2013 to February 2014 in five peasant associations (amba-1, amba-5, amba-14, Abrahamo, AAGRC), and Assosa town. During sampling sex, age, body condition, breed, management system and origin of animals were recorded. The body condition score was categorized as poor and good taking the middle point as a border in the 9 scale scores of method of body condition scoring for zebu cattle (Nicholson and Butterworth, 1986).

Questionnaire survey

Questionnaire survey was conducted to assess the knowledge and practices of farmers in the control of trypanosomiasis. To address the questionnaire survey, a total of 70 farmers in the 5 districts were interviewed with a structured questionnaire format. The interviewed people were selected randomly from the study areas.

Sample Size Determination and Sampling Method

The desired sampling size was calculated according to the formula given by Thrusfield (2005), with 28.1% excepted prevalence (Mulaw *et al*., 2010), at 95% confidence interval and 5% absolute precision. Accordingly, a total of 310 samples were collected during the study period using systematic random sampling technique. Sample size was proportionally distributed to kebeles.

Parasitological study

Packed Cell Volume (PCV) determination: Blood samples were obtained by puncturing the marginal ear vein with a lancet and collected directly into a pair of heparinized capillary tubes. The tubes were then sealed at one end with crystal seal. The capillary tubes were placed in micro-hematocrit centrifuge and were allowed to centrifuge at 12,000 revolutions per minute (rpm) for 5 minutes. After centrifugation, the capillary tubes were placed in a hematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anaemic (Murray, 1988).

Buffy Coat Technique: After the centrifugation, trypanosomes were usually found in or just above the buffy coat layer. The capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was expressed onto a glass slide, and covered with cover slip. The slide was examined under x40 objective and x10 eye piece for movement of parasite. Trypanosome species were identified according to their morphological descriptions on Giemsa stained blood film as well as movement in wet film preparations (Marquardt *et al*., 2000).

Data Management and Analysis

The data collected was entered into Microsoft Excel spread sheet. All analysis was performed using STATA statistical software package (Stata 11.0). P-value < 0.05 was considered to determine level of significance.

Result

Parasitological prevalence

Out of 310 blood samples, the overall prevalence of bovine trypanosomosis was found to be 22.9% (Table 1).

Table 1: Prevalence of bovine trypanosome infections in Assosa district on the basis of kebele/location.

Kebele	No of animals examined	No. positive	Prevalence (%)
AAGRC	20	1	5
Assosa	110	27	24.5
Amba-14	54	8	14.8
Abrahamo	18	7	38.8
Amba-5	54	14	25.9
Amba-1	54	14	25.9
Total	310	71	22.9

The most common trypanosome species identified were *T. congolense* 13.2% (41/310) followed by *T. brucei* 3.2% (10/310), *T. vivax* 2.9% (9/310), mixed infections of *T. vivax* and *T. congolense* 1.6% (5/310), *T. congolense* and *T. brucei* 1.29% (4/310) and *T. brucei* and *T. vivax* 0.64 (2/310) (Table 2).

Table 2: Prevalence of bovine trypanosome in Assosa district on the basis of Trypanosoma by species identified.

Kebele	Positive (%)	Trypanosoma identified					
		T. congolense	T. brucei	T. vivax	Mixed infections		
					T. congolense and T. vivax	T. congolense and T. brucei	T. brucei and T. vivax
AAGRC	1(5%)	1(5%)	0.00	0.00	0.00	0.00	0.00
Assosa	27(24.5%)	20(18.2%)	4(3.6%)	2(1.8%)	2(1.8%)	1(0.9%)	0.00
Amba-14	8(14.8 %)	3(5.5%)	1(1.8%)	1(1.8%)	1(1.8%)	1(1.8%)	1(1.8%)
Abrahamo	7(38.8%)	6(33.3)	1(5.5%)	0.00	0.00	0.00	0.00
Amba-5	14(25.9)	7(12.9%)	2(3.7%)	2(3.7%)	1(1.8%)	2(3.7%)	0.00
Amba-1	14(25.9)	4(7.4%)	2(3.7%)	4(7.4%)	1(1.8%)	0.00	1(1.8%)
Total	71(22.9%)	41(13.2%)	10(3.2%)	9(2.9%)	5(1.6%)	4(1.29%)	2(0.6%)

Among host and management related factors only body condition was found to be risk factor for trypanosome infection. Animals of poor body condition were 2.11 times more likely of getting trypanosome infection than animals in good body condition (Table 3).

Table 3: Host and management-related risk factors associated with the prevalence of trypanosome infections.

Risk Factor		No of examined	Prevalence (%)	Odds ratio	95% CI	*p-value*
Body condition	Good	261	54(20.7%)	1.00	-	
	Poor	49	17(35.4%)	2.11	1.09-4.09	0.027
Sex	Female	180	38(21.1%)	1.00	-	0.54
	Male	130	33(25.4%)	1.18	0.69-2.01	
Age	< 2 yrs.	40	13(32.5%)	1.00	-	0.131
	2-4 yrs.	144	31(10%)	0.56	0.26-1.19	0.300
	>4 yrs.	126	25(8%)	0.43	0.19-0.94	
Management system	Semi-intensive	20	1(5%)	1.00	-	
	Extensive	290	70(24.1%)	6.05	0.79-45.97	0.082

The mean PCV values and standard deviation (SD) for the whole examined animals was 25.03±5.5. However, the mean PCV value for aparasitaemic (non-diseased) animals was 25.8 ± 5.5 and mean PCV of Parasitaemic (diseased) animals were 22.3 ± 4.4 (Table 4). The difference was found to be statistically significant ($p<0.001$).

Table 4: Mean Packed Cell Volume in parasitaemic and aparasitaemic animals.

Status	Sample frequency	Mean PCV (%)	SD
Parasitaemic	71	22.3	4.4
Aparasitaemic	239	25.8	5.5
Total	**310**	**25.03**	**5.5**

SD- Standard deviation

Questionnaire Survey

All interviewed livestock owners (100%) know bovine trypanosomosis very well and locally call it "Wozwuz" or Gendi to say disease that causes wasting or emaciation. According to farmers response to the major diseases symptoms in relation to trypanosomosis, the following symptoms were listed according to their frequency of response; emaciation, weakness, rough hair coat, reduced milk production, depression, lacrimation and nasal discharge (Table 5).

According to farmers response the major signs in relation to trypanosomosis were recorded. According to farmer's frequency of response: rough hair coat, weakness, depression, reduced milk production, emaciation, lacrimation and nasal discharge were the major clinical signs. Concerning knowledge of disease transmission and season of occurrence, only 12.8% of the farmers responded that the tsetse flies are responsible, while 63% associates with cold weather and 24.2% do not know anything about the vector for trypanosomosis. The respondents consider season of occurrence of the disease to be from June to September (34.3%), July to December (25.7%) and September to November (40%) (Table 5).

Table 5: Findings of the questionnaire survey

No	Variables		Frequency of Response	Percentage (%)
1	Diseases symptoms in relation to trypanosomosis	Emaciation	70	100
		Weakness	58	82.8
		Rough hair coat	45	64.3
		Reduced milk production	38	54.3
		Depression	26	37.1
		Lacrimation	24	34.3
		Nasal discharge	17	24.3
	Disease transmission	Tsetse flies	9	12.8
		Cold weather	44	63
		Don't know	17	24.2
2	Season of occurrence	June to September	24	34.3
		September to November	28	40
		July to December	18	25.7
3	Control and prevention	Trypanocidal drugs	70	100%
		Nothing	-	-

Discussion

The study revealed that the trypanosomosis caused by different species of trypanosomes were common in Assosa district in domestic livestock's specially cattle's. The overall disease prevalence in the study area was 22.9% indicating that trypanosomes are still of much concern and represent a major obstacle to livestock production. This study agreed with findings of 28.1% (Mulaw *et al.*, 2010) in Assosa district, 24.7% (Ali and Bitew, 2011) in Mao Komo special district which were higher than the current study. The current result was relatively lower than the previous findings. This could be due to variation in study site, sample size, study season, extensive or seasonal clearing of bushes, expansion of human and agricultural investment in the area affecting the agro-ecology.

This study has also shown that *T. congolense* was the dominant species with a proportion of 13.2% followed by *T. brucei* 3.2%, *T. vivax* 2.9%, mixed infections

(*T. congolense* and *T. vivax* 1.6%, *T. congolense* and *T. brucei* 1.29%, *T. vivax* and *T. brucei* 0.6%). This result concords with the reported proportion of 63.2% *T. congolense*, 13.6% *T. vivax*, 11.6% *T. brucei* and 11.6% mixed infection in cattle from the neighboring Mao-Komo special district (Ali and Bitew, 2011). This result is also in agreement with the reported proportions of *T. congolense* (77.6%) followed by *T. vivax* (14.9%), *T. brucei* (6.0%) and *T. congolense* and *T. vivax* mixed infection (1.5%) from Metekel and Awi zones (Afework *et al*., 2000). The high ratio of *T. congolense* in tsetse-infested area may be ascribed to the more efficient transmission of *T. congolense* by major cyclical vectors than *T. vivax* in East Africa (Kuzoe, 1991). The difference in the infection rate may be explained by the variation in study site and duration, season of study and vegetation coverage. The highest and the lowest prevalence were recorded in Abrahamo (38%) and AAGRC (5%) respectively. However, there was no statistically significant difference ($p>0.05$) in the prevalence of trypanosomosis in the study sites, because they were located in similar agro-ecology of tsetse belt. The associations of the disease with age and sex were assessed. No statistically significant association was observed with respect to age and sex ($p>0.05$). Infection in poor body condition was significantly higher than good body conditions. This is finding is in agreement with a study report by Mehirt and Mamo, (2007). Out of infected animals examined 20.7% from good body condition and 35.4% from poor body condition were found positive of the trypanosomosis and they were statistically significant ($p<0.05$). This is in agreement with Afework *et al*., 2000. The mean PCV of parasitaemic animals is lower than mean PCV of aparasitaemic animals and, the difference was highly significant ($p<0.001$). This finding roughly in agreement with what was reported by Cherinet *et al.* (2004), Tadesse *et al.* (2011) and Zeryehun and Abraham (2012).

In order to control and prevent bovine trypanosomosis, knowledge of farmers should be assessed. Rapid method for assessing their knowledge and enhancing the control and prevention with active participation of livestock owners should be in place. Therefore, this survey was conducted with the objective of exploring the knowledge assessment of farmers in control and prevention of bovine trypanosomosis in Assosa district. According to farmers response, the season of occurrence of the disease considered to be June to September 24 (34.3%), July to December 18 (25.7%) and September to November 28 (40%).

Ability of livestock keepers to determine seasonal variations and peak level of trypanosomosis and its vectors as recorded in the present study is in line with

the reports of Chernet *et al*. (2004); Dagnachew *et al*. (2005) and Tesfaye *et al*. (2001) from Ethiopia, Catley *et al*. (2009) from Kenya and Grace *et al*. (2009) from west Africa. Wet and warm months of the year (such as May and June) are favorable periods for vectors and trypanosomes growth and multiplication and are observed as peak months of infection). The way of transmission of trypanosomosis is not well known by most of farmers and they have no idea (24.2%), while some consider as to be following cold season (63%). However, a few knows as by tsetse flies (12.8%). This finding disagreed with recent reports showing significantly higher numbers of respondents in the study districts are aware of the causal association between biting flies and bovine trypanosomosis but not considered as the etiological factor. This coupled with their knowledge on the signs of the disease and treatment suggests that the farmers have comparable understanding of the problem as reported earlier (Maudlin *et al*., 2004; Catley *et al*., 2009) in other African countries.

In conclusion, animal trypanosomosis is a major obstacle to livestock production and productivity in Assosa district. In this study only few livestock owners know the vector (tsetse) association with the disease. However, most of them have satisfactory knowledge in suggesting signs of trypanosomosis and its impact on production and they have strong desire to give their part on control and prevention of trypanosomosis. The prevalence of trypanosomosis found in the study area is high. Thus, further studies on the distribution of tsetse flies and trypanosomosis should be conducted throughout all seasons of the year. Awareness creation on vector (tsetse) control by the farmers by providing trainings and materials for trapping is very crucial.

References

Afework, Y., Clausen, P.H., Abebe G, Tilahun, G., Mehlitz, D., 2000. Multiple-drug resistant Trypanosome congolense populations in village cattle of metekel district, north-west Ethiopia. *Acta Trop*., 76(3), 231-238.

Ali, D. and M. Bitew, 2011. Epidemiological study of bovine trypanosomosis in Mao-Komo special district, Benishangul Gumuz, Regional State, Western Ethiopia. *Global Veterinaria*, 6(4), 402-408.

Catley A, Irungu P, Simiyu K, Dadye J, Mwakio W, Kirangu J, Nyamwaro SO.,2009. Participatory investigations of bovine trypanosomosis in Tana River district, Kenya. *Med. Vet. Entomol.,* 16 (1), 55-66

Chernet T, Sani RA, Panandam JM, Nadzr S, Speybroeck N, Van den Bossche P., 2004. Seasonal prevalence of bovine trypanosomosis in a tsetse-infested zone and

a tsetse-free zone of the Amhara Region, Northwest Ethiopia. *Onderstepoort J. Vet.* Res., 71, 307–312.

CSA (Central Statistical Authority), 2005. Agricultural Sample Survey, Statistical Bulletin331, Addis Ababa, Ethiopia, 85, 391-404.

CSA (Central Statistical Authority), 2008. Federal Democratic Republic of Ethiopia, Central Statistical Agency, Agricultural Sample Survey, Statistical Bulletin 417, Addis Ababa, Ethiopia, pp 44.

Dagnachew, S., A.K. Sangwan and Abebe, G., 2005. Epidemiology of bovine trypanosomosis in the Abay (Blue Nile) basin areas of northwest Ethiopia. *Rev. Med. Vet.*, 58(3):151-157.

Desta, M., Menkir, S., Kebede, A., 2013. The study on tsetse fly (*Glossina species*) and their role in the trypanosome infection rate in Birbir valley, Baro Akobo River system, western Ethiopia. *J. Vet. Med. Anim. Health.* 5(7), 186-194.

ETTA (Ethiopia Agricultural Transformation Agency), 2014.

FAO, 1998. A field guide for the diagnosis, treatment and prevention of African Trypanosomes: In Food and Agriculture organization of the United Nations, Rome, Italy, Pp. 170.

Feysa, F., 2004. Current Epidemiology situation of bovine trypanosomiasis in limu shaye tsetse control areas of upper Didessa valley. MSc, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

Abebe, G., 2005. Trypanosomosis in Ethiopia. *SINET Ethiopia J. Sci*, 27(1), 1-8.

Grace, D., Randolph, T., Affognon, H., Dramane, D., Diall, O., Clausen, P.H., 2009. Characterization and validation of farmers' knowledge and practice of cattle trypanosomosis management in the cotton zone of West Africa. *Acta. Trop.*, 111(2), 137-143.

Kuzoe, F.A.S., 1991. Perspectives in research and control of African trypanosomiasis. *Ann.Trop. Med. Parasitol.*, 85(1), 33-41.

Maudlin I, Holmes PH, Miles MA., 2004. The Trypanosomiasis. CAB International Publishing, Wallingford, UK, pp. 1-634.

Mihret, A., and Mamo, G.,2007. Bovine Trypanosomosis in three districts of East Gojjam Zone bordering the Blue Nile River in Ethiopia. *J. Infect. Dev. Ctries.*, 1(3), 321-325

Mulaw, S., Addis, M., Fromsa, A., 2010. Study on the Prevalence of Major Trypanosomes Affecting Bovine in Tsetse Infested Assosa District of Benishangul Gumuz Regional State, Western Ethiopia, *Global Vet.*,7 (4), 330-336.

Nicholson, M.J., and Butterworth, M.H., 1986. A Guide to Condition Scoring of Zebu Cattle. International Livestock Centre for Africa. Addis Ababa, Ethiopia, pp. 29.

Murray, M., Dexter, T.M., 1988. Anemia in bovine Africa Trypanosomosis. *Acta Trop.,* 45, 389-432.

Marquardt, W.C, Demaree, R.C., and Grieue, R. B., 2000, Parasitology and vector biology, 2nd edn. Academic Press, London, UK.

Tadesse, A., Hadgu, E., Mekibib, B., Abebe, R., Mekuria, S., 2011. Mechanically Transmitted Bovine Trypanosomosis in Tselemty Woreda, Western Tigray, and Northern Ethiopia, *J. Agri.*, 6(1),10-13.

Tesfaye, D, Speybroeck, N., Deken, R.D, Thys, E., 2001. Economic burden of bovine trypanosomosis in three villages of Metekel zone, Northwest, Ethiopia. *Trop. Anim. Hlth, Prod*., 44(4), 873-879.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd ed., Blackwell Science Ltd, UK, pp. 233-250.

Torr, S.J., Wilson, P.J., Schofield, S., Mangwiro, T.N.C., Akber, S.,White, B.N., 2001. Application of DNA markers to identify the individual-specific host of tsetse feeding on cattle. *Med. Vet. Entomol*., 15(1),78–86.

Uilenberg G. (1998). A Field Guide for the Diagnosis, Treatment, and Prevention of African Animal Trypanosomosis. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. pp 170.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1996. Veterinary Parasitology, 2nd ed. Blackwell, Science Ltd.

Wikipedia., 2005. The free encyclopedia.

Zeryehun, T., and Abraham, Z., 2012. Prevalence of Bovine Trypanosomosis in Selected District of Arba Minch, SNNPR, Southern Ethiopia. *Global Vet.*, 8 (2),168-173.

Assessment of retrospective rabies suspected cases registered at two hospitals, community and traditional healers' knowledge, attitude and practices in south Ethiopian pastoralist.

*Balako Gumi [*1,2] Sisay Girma[2], Hussein Mohamed [2], Oudessa Kerro[3] and Hassen Chaka [4]*

[1]Addis Ababa University, College of Veterinary Medicine & Agriculture, Department of Microbiology & Veterinary Public Health, P.O. Box 34, Bishoftu, Ethiopia

[2]Bule Hora University, College of Agriculture, P.O. Box 144, Bule Hora, Ethiopia

[3]The University of Tennessee, Department of Animal Science, Knoxville, TN 37996, USA

[4]Food and Agricultural Organization of united nations, Addis Ababa, Ethiopia

**Corresponding author: Tel: +251930069300. E-mail address: balako.gumi@yahoo.com.*

Abstract

Despite of available protective rabies vaccines for both human and animal, transmission of rabies from animals to people continues in Africa and Asia. Every year, over 59,000 people are estimated to die from the disease globally. Most of industrialized countries in Europe and North America have eliminated rabies from domestic dog populations. However, in the majority of developing countries, rabies remains endemic in domestic dog and poorly controlled. To understand rabies situation in pastoralist and agro-pastoralists area of southern Ethiopian, retrospective data on 431 rabies suspected animal bite cases registered at Bule Hora and Yabello Hospitals were collected and reviewed. Moreover, the knowledge, attitude and practice of 107 selected community members and 55 traditional healers were assessed. Out of 431 cases, 55.7% and 24.4% were ≤ 15 and between 16-25 years age group, respectively. There were more male 55.5% cases than female 44.5%. Only 32% of cases registered at Yabello Hospital received post exposure prophylaxis (PEP), whereas the remaining referred to other health facilities. Ninety eight percent (98 %) of animal responsible for the bite was dog and the remaining were cat, fox and donkey. Most of bites were occurred in March-June (36.2%) and November-February (35.5%). Out of interviewed participants 87.8% of them know rabies and 83.2% were able to mention rabid animals' symptoms. The 78.5% of participants did mention how rabies transmitted to dog and 88.1 % of them described dog as main source of infection. The 91.6% of participants told us that rabies is transmit-

ted to human by rabid animals' bite and 94.3% of them believe that rabies is treatable disease and the major means of prevention is traditional treatment. Almost all traditional healers mention that the symptom of rabies in human and animals, and its way of transmission; however 90.9% of them believe that traditional treatment as effective means of rabies control. Interview result of both community members and traditional healers indicated that most of the rabies cases treated by the traditional healers. Health service providers have to make PEP vaccines available for bite victims and need to raise awareness of the local communities through health education about rabies and animal bite management.

Keyword: Retrospective Study; Rabies; South Ethiopia; pastoralists

Introduction

Rabies is one of the highly fatal zoonosis, but globally neglected, more specifically in developing countries (Knobel *et al*., 2005). The disease is caused by a negative-stranded RNA virus of the *Rhabdovirdae* family (Dietzschold *et al*., 2005)a negative-stranded RNA virus of the rhabdovirus family. RV pathogenesis, like that of other viruses, is a multigenic trait. Recent findings indicate that in addition to the RV G protein viral elements that regulate gene expression, especially expression of the L gene, are also likely to play a role in RV pathogenesis. In vivo, RV infects almost exclusively neurons, and neuroinvasiveness is the major defining characteristic of a classical RV infection. A key factor in the neuroinvasion of RV is transsynaptic neuronal spread. While the ability of RV to spread from the post-synaptic site to the pre-synaptic site is mediated by the RV G protein, the RV P protein might be an important determinant of retrograde transport of the virus within axons. Although the mechanism(s and a fatal disease that affects domestic and wild animals, and is spread to people through close contact with infectious material, usually saliva, via deep bites or scratches. Once the symptoms of the disease have developed, rabies is nearly always fatal. Dogs are the main host and transmitter of rabies (Vodopija *et al*., 2016). Despite of available protective rabies vaccines for both human and animal, transmission from animals to people continues. In Africa and Asia, more than 59,000 people die annually from the disease (Léchenne *et al*., 2016; Zinsstag *et al*., 2017). Most of industrialized countries in Europe and North America have eliminated rabies from domestic dog populations (Hampson et al., 2015). A concerted effort by South and Central American countries has reduced dog rabies transmission close to elimination (Hampson et al., 2007).

However, in the majority of developing countries, rabies remains endemic in domestic dog and poorly controlled (Hampson et al., 2015).

Ethiopia has one of the highest incidence levels of human rabies in Africa, with 3–7 deaths per 100,000 people annually (Beyene et al., 2018). Deressa et al 2010 reported 35-58 annual human death due to rabies in Addis Ababa and its surrounding during 2001-2009 (Deressa et al., 2010). The impact of rabies in Ethiopia is aggravated due to limited availability, accessibility, affordability, awareness and knowledge of rabies PEP after exposure to rabid dogs and other animals (Jemberu et al., 2013). In addition to health impact the disease also affect livelihood through losses of valuable livestock and cost of travel to health center for PEP treatment (Yimer et al., 2002). Jibat et al estimated annual national economic losses of 209 million USD due to rabies in cattle (Jibat et al., 2016) and on average 2 million USD treatment costs for PEP in Ethiopia (Beyene et al., 2018).

In Ethiopia, the majority of rabies cases are diagnosed on clinical symptoms only, attributed to the distance to centrally located diagnostic facility at Ethiopian Public Health Institute, and the absence of a reliable laboratory-based surveillance system in the rest of country. Consequently, rabies case reporting rate from urban and rural sectors are low. The majority of exposed patients use traditional healers, in particularly in the rural areas. The limited available data indicates that it is the disease of public health, economic and social concern (Jibat *et al.*, 2016). Given the uncertain rabies situation in the Ethiopian, specifically in West Guji and Borena Zones, it was an important to assess the situation of the disease in the areas as well as knowledge, attitude and practice of the people towards rabies in general. The aim of study was to contribute to better understanding of situation of the disease that would, in turn assist control measures. Therefore, the case records of rabies suspected animal bites were retrospectively assessed in Bule Hora and Yabello Hospitals in southern Ethiopia. Knowledge, attitude and practice of the people and traditional healers about rabies were also assessed.

Materials and Methods

The study area

Retrospective study was conducted in 2016, in West Guji and Borena Zones of Oromia regional state in south Ethiopia. The zonal capitals, Bule Hora and

Yabello, are located at 470 and 560 km from Addis Ababa, respectively (Fig. 1). The two zones has an area of 45,435 square kilometers, a human population of 1.15 million and a livestock population of 1.13 million cattle, 1.06 million small ruminants, 0.072 million equines and 0.1 million camels (CSA, 2011).

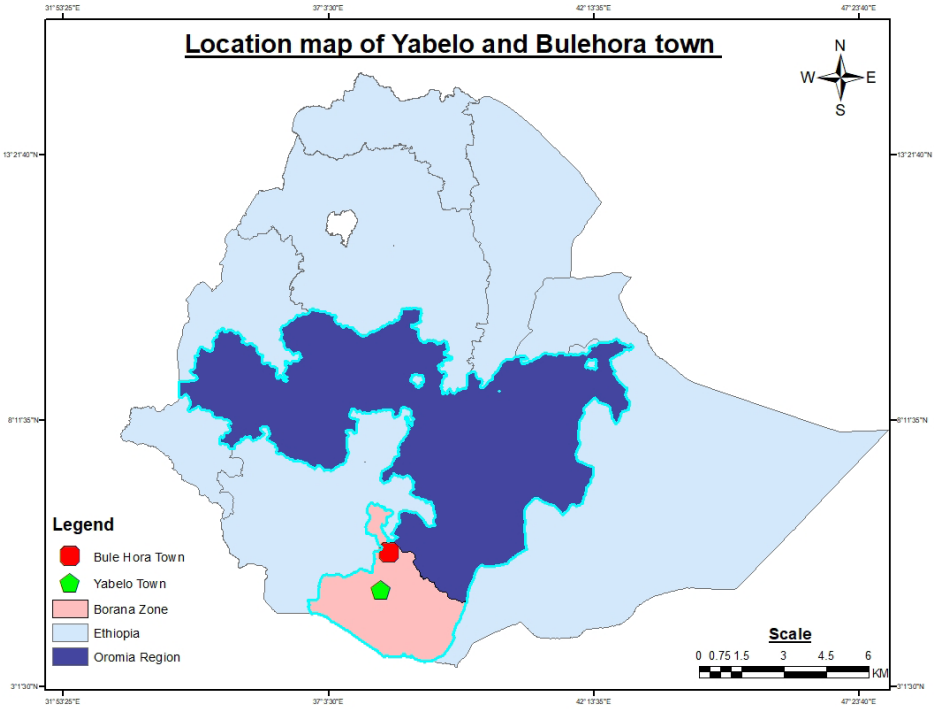


Figure 1: Map of Yabello and Bule Hora town

Sampling methods

Bule Hora and Yabello districts were selected purposively due to availability of hospitals from West Guji and Borena zones, respectively. There were only two hospitals in the two zones. Cases registered from November 2014-June 2016 at Yabello and March 2015 – September 2016 at Bule Hora Hospitals were reviewed. A predesigned format was used to collect data that basically establish age and sex profiles of patients, patient management at hospitals, if died case report, season of case report, animal responsible for bite. Two hundred sixty-eight and 163 rabies suspected case records were reviewed from Yabello and Bule Hora Hospitals, respectively.

Twenty-three and 19 Kebeles (a lowest government administrative unit) were randomly selected from list of kebeles in Bule Hora and Yabello district, respectively. In Bule Hora, 3 urban and 20 rural kebeles were included, while all 19 kebeles in Yabello district were from rural areas. Data was collected through a structured questioner survey. Questionnaire was translated into *Afaan Oromo* (a local language), and interview was made in *Afaan Oromo*. One hundred seven (107) voluntary community members and 55 traditional healers were interviewed. The questionnaires address the respondents' knowledge about rabies (causes, means of transmission, treatment after animal bite and prevention methods). The questionnaires for traditional healers explore their knowledge about rabies and how they handle rabies cases. All participants gave their oral informed consent before interview.

Data management and analysis

Data collected were coded and entered into Microsoft Excel spreadsheet, double checked with questionnaire information to avoid errors and then cleaned data import to STATA. Descriptive statistics such as frequency and percentage were used to summarize the data.

Result

Among 431 rabies suspected animal bite cases 55.7% were children (age $\leq$ 15 years) and 24.4% young (16-25 years) age group. There was more male 55.5% cases than female 44.5%. Two hundred sixty-eight cases registered at Yabello Hospital, only 32% were received PEP, whereas all cases registered at Bule Hora Hospital received PEP. Out of animals responsible for the bites, 98% were dogs and the remaining were cat, fox and donkey. Over all cases registered at two hospitals, 67.7% of them were received PEP, while 32.3% was made wound treatment and referred to other health facilities. The majority of cases were occurring in summer (36.2%) and winter (35.5%) seasons (Table 1).

Table 1: Rabies suspected animal bites registered at Bule Hora and Yabello Hospitals

Variables	Categories	Frequency (%)	P-Value
Hospitals(N=431)	Bule Hora	163 (37.8)	
	Yabello	268 (62.2)	
Gender	Male	239 (55.5)	0.262
	Female	192 (44.5)	
Cases by age categories (N=431)	≤15	240 (55.7)	0.118
	16-25	105 (24.3)	
	26-35	37 (8.6)	
	>35	49 (11.4)	
Animals responsible for bite (N=405)	Dog	397 (98)	0.001
	Cat	2 (0.5)	
	Fox	1 (0.3)	
	Donkey	5 (1.2)	
Care given at hospitals (N=368)	PEP (Bule Hora=163, Yabello=86)	249 (67.7)	
	Referred (Bule Hora=0, Yabello=119))	119 (32.3)	
Cases by four seasons(N=431)	Mar-June (major rain)	156 (36.2)	0.001
	July-August (minor dry season)	63(14.6)	
	Sept-Oct. (minor rain season)	59(13.7)	
	Nov-Feb (Major dry season)	153(35.5)	
Rabies suspected case death report (N=431)	Death*	1 (0.23)	

*Death report mentioned in this retrospective study was not confirmed by laboratory diagnosis and only based on clinical diagnosis.

The knowledge and practices of 107 interviewed participants revealed that 87.8% of them has knowledge of dog rabies while, 12.3% has no knowledge of dog rabies. Seventy two percent (72%) of the participant own dogs and uses dog as house or livestock guard. Out of 107 participants, 83.2% were able to mentioned rabid animals' symptoms, while the remaining 16.8% unable to describe rabies symptom. The 78.5% of participants were mentioned how rabies transmitted to dog and 88.1 % of them described a dog as main source of infection. Most of the participants (91.6%) mentioned that rabies transmitted to human by rabid animals' bites, 5.6% described as eating raw meat transmit

rabies, while the remaining 2.8% didn't know how rabies transmit. Thirty-eight-point three percent (38.3%) of interviewed participants didn't know the reservoir of rabies, while the remaining participants mentioned either dog or other animals as reservoirs. Among interviewed participants 59.8% of them; their family members were exposed to rabid animals during the last 10 years. The family members exposed to bites, 43.5% of them exposed more than two times. About 73.4% of victims were treated by traditional healers. Most of victims were children 64.7% and women 23.5%. The 94.3% of participants believe that rabies is a curable disease. Among 66.4% of participants who believe that human rabies is preventable; they mentioned that the prevention is made by traditional treatment 60.6%, killing rabid animals 14.1%, dog control 7% and by avoiding contact with rabid animals 2.8% (Table 2).

The interview result of traditional healers revealed that 80.4% of them were illiterate (can't read and write) and 54.7% of them has more than 20 years of traditional healers' services. The 58.5% (31/53) participants mentioned that dog as the animal affected by rabies, while, 41.5% of them mentioned that rabies can affect all animal species. Almost all participants mentioned that one or more symptoms of rabies in animals and human (Table 3). Almost all (98.2%) of participants mentioned that rabies as a treatable disease and 90.9% of them believe that the traditional treatment is an effective control method for rabies. Among 87.3 % of the traditional healers who had opportunity to treat rabies suspected exposure cases they clam 93.8 % of them as successful and 6.2 % as failure (Table 3).

Table 2: Knowledge, Attitude and Practice of 107 interviewed participants.

Variables	Categories	Frequency (%)	
		Yes	No
Participants by District (N=107)	Bule Hora	66 (61.7)	
	Yabello	41 (38.3)	
Own dog(s) (N=107)		77 (72)	30 (28)
Purpose to keep dog (N=77)	House/livestock guard	77 (100)	
Know dog rabies (N=107)		94 (87.8)	13 (12.2)
Rabies symptom (N=89)	prodromal	4 (4.5)	
	furious	38 (42.7)	
	paralytic & furious	47 (52.8)	
From where dog acquired rabies (N=84)	Dog	74 (88.1)	
	Other animals	7 (8.3)	
	Environment	3 (3.6)	
Reservoir of dog rabies (N=107)	Dog	45 (42.1)	
	Other animals	21 (19.6)	
	Don't know	41 (38.3)	
How rabies transmits to human (N=107)	Biting by rabid animal	98 (91.6)	
	Eating raw meat	6 (5.6)	
	Don't know	3 (2.8)	
Experience of family member bitten by animals in last 10 years (N=107)		64 (59.8)	43 (40.2)
Frequency of bite (N=62)	At least once	35 (56.5)	
	≥ 2times	27 (43.5)	
Where did Victim treated (N=64)	PEP*	16 (25)	
	Traditional medicine	47 (73.4)	
	local wound treatment	1 (1.6)	
Most victim dog/animal bite (N=102)	Men	8 (7.8)	
	Women	24(23.5)	
	men and women	2(2)	
	Children	66 (64.7)	
	children and women	2(2)	
Is human rabies treatable (N=106)		100 (94.3)	6 (5.7)
How human rabies can be prevented (N=71)	Vaccination	11 (15.5)	
	Traditional treatment	43 (60.6)	
	Dog control	5 (7)	
	Avoid contact with rabid animals	2 (2.8)	
	Kill rabid animals	10 (14.1)	

*PEP= Post exposure prophylaxis

Table 3: Knowledge, Attitude and Practice of 55 interviewed traditional healers.

Variables	Categories	Frequency (%)	
		Yes	No
Participant by district (N=55)	Bule Hora	42 (76.4)	
	Yabello	13 (23.6)	
Education status (N=51)	Illiterate (can't read & write)	41 (80.4)	
	Elementary (grade 1-8)	8 (15.7)	
	High school (9-12)	2 (3.9)	
Service year as healer (N=53)	<10	10 (18.9)	
	10-20	14 (26.4)	
	>20	29 (54.7)	
Animal affected by rabies (N=53)	dog	31 (58.5)	
	All animal species	22 (41.5)	
Rabies symptom in animals (N=55)	Prodromal	6 (10.9)	
	Furious	6 (10.9)	
	Paralytic	43 (78.2)	
Rabies symptom in human (N=54)	Anxiety	52 (96.3)	
	Confusion	2 (3.7)	
Is rabies treatable (N=55)		54 (98.2)	1(1.8)
Treatment for rabies(N=55)	Traditional treatment	50 (90.9)	
	Modern	5 (9.1)	
Experience to treat rabies cases (N=53)		49 (92.5)	4(7.5)
outcome of treatment (N=48)	Cured	45 (93.8)	
	death	3 (6.2)	

Discussion

In the present retrospective case study, among rabies suspected animal bite In the present retrospective case study, among rabies suspected animal bite cases, 55.7% were children while 24.4% young age group. There were more male cases than female, 55.5% and 44.5%, respectively. Comparable reports were from Oman, 70% male cases, and 26% of 10-19 age group (Abaidani et al., 2015), 62.8% male cases and 38.5% of cases were children in Ethiopia (Yibrah

and Damtie, 2015). Teklu et al from Ethiopia was reported more male cases (63%) and children (63%) (Teklu et al., 2017). More male exposure to animal bite may be due to the fact that men tends to approach or handle animals with strange behavior, while children are more friend with pet when they are healthy and easily exposed to bite when rabid.

Among animal species responsible for the bites 98% was due to dog, while the remaining were cat, fox and donkey. Different report was from Oman where most common bites from cats (Abaidani et al., 2015). This difference may be due to cultural and religion differences to keep dog as pet animal. All cases registered at Bule Hora Hospital received PEP; whereas only 32% of cases registered at Yabello Hospital received PEP. Beyene et al (2018) reported similar finding from Yabello district that among 87 suspected victims only 28.7% were received PEP (Beyene et al., 2018). This low proportion of access to PEP was due to PEP vaccine unavailability. Most of the cases were occurred in main rainy and main dry seasons.

There was similar report from Amhara region of Ethiopia (Yibrah and Damtie, 2015). These seasonal differences may be due to the dogs' seasonal activities, such as breeding seasons in rainy season and dogs' migration to urban village for food scavenging in the dry season.

In the present study 87.8% of interviewed participants have knowledge of dog rabies and 72% of them use dog as guarding house or their livestock. When livestock guarding dog get rabid it can bite lot of village livestock and herding children before family recognized the disease. Among 107 participants, 83.2% of them mentioned rabies symptom and 78.5% of them described how dog contract the disease. The 91.6% of the participants mentioned that rabies transmitted to human by rabid animal bites. The comparable result was reported from Addis Ababa and northern Ethiopian (Ali et al., 2013; Jemberu et al., 2013) and Tanzania (Sambo et al., 2014). Regarding practice and exposed family members' managements, 73.4% of them were treated by traditional healers. Jemberu et al reported higher rate of victims treated by traditional healers in north Ethiopia than present result (Jemberu et al., 2013). This difference may be due to level of awareness or trust on traditional healers than modern medicine in the northern Ethiopia.

Among the interviewed 55 traditional healers, 80.4% of them were illiterate (can't read & write) and almost all of them mentioned rabies symptom and

as treatable disease. Most of traditional healers, 90.9% of them believe that traditional treatment is an effective control method. This high level of belief on traditional treatment may be due to a knowledge on disease causing agent or healers wants to keep their respect in the community and healing/treating as source of income and economic benefit. Almost all traditional healers use the plants for both human and animals healing purposes. Among 87.3 % of the traditional healers who had opportunity to treat rabies suspected exposure cases 93.8 % of them reported as successful while 6.2 % as failure. However, the traditional healers claim such high rate of their treatment success, it must be handled with care in such a fatal disease unless it supported by scientific evidence. Moreover, cases they report may be bite victim than clinical cases.

Among 71.3% of rabies suspected animal bites, who registered at Yabello Hospital didn't receive PEP vaccine and further referred to other health providing facilities. Those victims were exposed to additional cost of travel and accommodation at treatment centers. Furthermore, it can delay start of PEP time and can increase the risk of onset of clinical rabies. Both interview result of 107 participants and 55 traditional healers indicated that most of rabies suspected animals bite victims rely on traditional healers' treatment and trust them than the government health providers. The local communities trust and dependency on traditional healers than the government may be due to strong cultural linkage with traditional healers and government service unavailability in the area. Therefore, this needs attention of regional and local health service providers to make available vaccines for PEP and create awareness through health education on rabies for local communities. Further study is needed to include more area coverage and retrospective study.

The limitations of present study were; retrospective rabies data on animal was not available, reported human death case in this study was not confirmed by laboratory diagnosis and only based on clinical diagnosis.

Acknowledgement

We would like to thanks Bule Hora University for financial support to research work and Bule Hora and Yabello Hospitals for the cooperation to review case records. We would like to thanks also interview participants for their cooperation and their time for face to face interview.

Funding source

The financial support for this retrospective study was obtained from the Bule Hora University research budget, Bule Hora Ethiopia.

Compliance with ethical standards

For questionnaire survey participants were briefed about the objectives of the study. All participants gave their oral informed consent before the interview.

Conflict interest

The authors declare that there is no conflict of interest.

References

Abaidani, I.A., Abri, S.A., Prakash, K.P., Hussain, M. Hassan, Hussain, M. Hammad and Rawahi, A.H.A., 2015. Epidemiology of rabies in Oman: a retrospective study (1991-2013). *Eastern Med. Health J.*, 21(8), 591–597

Ali, A., Ahmed, E.Y. and Sifer, D., 2013. A Study on Knowledge, Attitude and Practice of rabies among residents in Addis Ababa, Ethiopia. *Ethiop. Vet. J.*, 17, 19–35

Beyene, T.J., Mourits, M.C.M., Kidane, A.H. and Hogeveen, H., 2018. Estimating the burden of rabies in Ethiopia by tracing dog bite victims. *PloS One*, 13, e0192313

CSA, 2011. Central Statistical Agency Report, (Addis Ababa, Ethiopia)

Deressa, A., Ali, A., Beyene, M., Newaye, B., Yimer, E. and Hussen, K., 2010. The status of rabies in Ethiopia: A retrospective record review Ethiop. *J. Health Dev.*, 24, 127–132

Dietzschold, B., Schnell, M. and Koprowski, H., 2005. Pathogenesis of rabies Current Topics in Microbiology and Immunology, 292, 45–56.

Hampson, K., Coudeville, L., Lembo, T., Sambo, M., Kieffer, A., Attlan, M., Barrat, J., Blanton, J.D., Briggs, D.J., Cleaveland, S., Costa, P., Freuling, C.M., Hiby, E., Knopf, L., Leanes, F., Meslin, F.-X., Metlin, A., Miranda, M.E., Müller, T., Nel, L.H., Recuenco, S., Rupprecht, C.E., Schumacher, C., Taylor, L., Vigilato, M.A.N., Zinsstag, J., Dushoff, J. and Global Alliance for Rabies Control Partners for Rabies Prevention, 2015. Estimating the global burden of endemic canine rabies. *PLoS Negl. Trop. Dis.*, 9, e0003709.

Hampson, K., Dushoff, J., Bingham, J., Brückner, G., Ali, Y.H. and Dobson, A., 2007. Synchronous cycles of domestic dog rabies in sub-Saharan Africa and the impact of control efforts Proceedings of the National Academy of Sciences of the United States of America, 104, 7717–7722

Jemberu, W.T., Molla, W., Almaw, G. and Alemu, S., 2013. Incidence of rabies in humans and domestic animals and people's awareness in North Gondar Zone, Ethiopia. *PLoS Negl. Trop. Dis*., 7, e2216

Jibat, T., Mourits, M.C.M. and Hogeveen, H., 2016. Incidence and economic impact of rabies in the cattle population of Ethiopia. *Prev. Vet. Med*., 130, 67–76

Knobel, D.L., Cleaveland, S., Coleman, P.G., Fèvre, E.M., Meltzer, M.I., Miranda, M.E.G., Shaw, A., Zinsstag, J. and Meslin, F.-X., 2005. Re-evaluating the burden of rabies in Africa and Asia. *Bull.World Health Organ*., 83, 360–368

Léchenne, M., Oussiguere, A., Naissengar, K., Mindekem, R., Mosimann, L., Rives, G., Hattendorf, J., Moto Daugla, D., Alfaroukh, I., Zinsstag, J. and Technical Committee, 2016. Operational performance and analysis of two rabies vaccination campaigns in N"Djamena, Chad. *Vaccine*, 34, 571–577

Sambo, M., Lembo, T., Cleaveland, S., Ferguson, H.M., Sikana, L., Simon, C., Urassa, H. and Hampson, K., 2014. Knowledge, attitudes and practices (KAP) about rabies prevention and control: a community survey in Tanzania. *PLoS Negl. Trop. Dis*., 8, e3310

Teklu, G.G., Hailu, T.G. and Eshetu, G.R., 2017. High Incidence of Human Rabies Exposure in Northwestern Tigray, Ethiopia: A Four-Year Retrospective Study. *PLOS Negl. Trop. Dis.*, 11, e0005271

Vodopija, R., Racz, A. and Pahor, Đ., 2016. The incidence of Jackal bites and injuries in the Zagreb anti rabies clinic during 1995-2014 period. *Acta Clin. Croat*., 55, 151–155

Yibrah, M. and Damtie, D., 2015. Incidence of human rabies exposure and associated factors at the Gondar Health Center, Ethiopia: a three-year retrospective study. *Infect. Dis. Poverty*, 4(1), 3

Yimer, E., Newayeselassie, B., Teferra, G., Mekonnen, Y., Bogale, Y., Zewde, B., Beyene, M. and Bekele, A., 2002. Situation of Rabies in Ethiopia: A retrospective study 1990-2000. *Ethiop. J. Health Dev*., 16(1), 105–112

Zinsstag, J., Lechenne, M., Laager, M., Mindekem, R., Naïssengar, S., Oussiguéré, A., Bidjeh, K., Rives, G., Tessier, J., Madjaninan, S., Ouagal, M., Moto, D.D., Alfaroukh, I.O., Muthiani, Y., Traoré, A., Hattendorf, J., Lepelletier, A., Kergoat, L., Bourhy, H., Dacheux, L., Stadler, T. and Chitnis, N., 2017. Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure. *Sci. Transl. Med*., 9

Abomasal nematode parasites in goats slaughtered in Mekelle town, northern Ethiopia

*Kassaye Berhe and Kassaye Aragaw**

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

**Corresponding author. Tel.: +251924456196; E-mail address: kassaye.a@gmail.com (K. Aragaw).*

Abstract

Infection with abomasal nematodes, especially haemonchosis, is one of the most important problems challenging small ruminant production in the tropics. This study was carried out to identify nematodes infecting the abomasa, and estimate their prevalence and count in goats slaughtered in Mekelle town, northern Ethiopia. A total of 166 abomasa of goats were examined for post-mortem differential adult nematode parasites count using standard procedure. *Haemonchus* spp. and *Trichostrogylus axei* were recovered from 126 (75.9%) and 109 (65.7%) abomasa, respectively. It was noted that 152 (91.6%) goats harbored at least one of the parasites, while 83 (50%) goats were found infected with both parasites. Mean and maximum adult worm counts were 39.2 and 270 and 55.2 and 600 for *Haemonchus* spp. and *T. axei,* respectively. Months of the year had significant (p<0.05) effect on prevalence and adult worm count in both species of parasites identified. The adult worm count and prevalence were relatively high in February for *Haemonchus* spp. and in December for *T. axei.* The high prevalence of these economically important parasites in goats in the dry season may entail insidious losses they could incur in the productivity of goats in the study area.

Keywords: Abomasal nematodes; Ethiopia; Goat; *Haemonchus; Trichostrogylus axei*

Introduction

Goats in Ethiopia, with estimated population of about 30.2 million (CSA, 2017), makeup a significant proportion of the country's ruminant livestock inventory. They are integral part of the mixed crop-livestock system commonly practiced in the highlands of the country and the pastoral and agro-pastoral systems of the arid and semi-arid lowlands (Tolera and Abebe, 2007; Legesse *et al*., 2010; Kebede *et al*., 2012). Goats considerably contribute to household income and

nutrition of rural population of Ethiopia, and export earnings of the country (FAO, 2004; Tolera and Abebe, 2007; Legesse *et al*., 2010).

Infections with gastrointestinal (GI) nematodes remain one of the most prevalent parasitic diseases affecting small ruminants worldwide (Torres-Acosta and Hoste, 2008). They cause considerable economic loss mainly due to insidious production loss in addition to clinical diseases and mortalities they cause (Over *et al*., 1992). GI nematode parasites, especially haemonchosis, are significant causes of loss of productivity and mortality in small ruminants in Africa (Allonby and Urquhart, 1975; Githigia *et al*., 2001). Though not substantially supported by studies quantifying losses, researchers have agreed that nematode infections are among the most devastating diseases of small ruminants in Ethiopia with huge economic significance (Asmare *et al*., 2016).

Surveys conducted in different places in Ethiopia (Abebe and Esayas, 2001; Woldemariam, 2005; Menkir *et al*., 2007; Aga *et al*., 2013; Haile *et al*., 2018) and elsewhere in Africa (Githigia *et al*., 2001) demonstrated that *Haemonchus* spp., are generally the most dominant GI nematode parasites in goats. Other commonly reported groups of abomasal parasites in goats in Ethiopia include: *Trichostrongylus axei* and *Ostertagia/Teladorsagia* spp. (Abebe and Esayas, 2001; Kumsa and Wossene, 2006; Bitew *et al*., 2011; Aga *et al*., 2013; Teshale and Aragaw, 2014).

The development of cost-effective and sustainable control program to helminth infections requires thorough knowledge of the species of parasites present in an area among others (Hansen and Perry, 1994). Therefore, this study was conducted to identify nematodes infecting the abomasa, and estimate their prevalence and intensity of infection in goats slaughtered in Mekelle town in northern Ethiopia.

Materials and Methods

Study area

The study was conducted in Mekelle town, the capital of Tigray Region, located about 783 km north of Addis Ababa. The area lies between 13°32′ and 13°30′N latitude and 39°29′ and 39°39′ E longitude and covers a total area of 3500 hectares in the northern highlands of Ethiopia (Fig. 1). The elevation of the city measures between 2000 and 2270 m.a.s.l. Agro-climate of the area is semi-arid

with average minimum and maximum temperature of 17.6 and 24.3°C, respectively and annual rainfall of 700 mm (TBOANR, 1999; MCPPP, 2007).

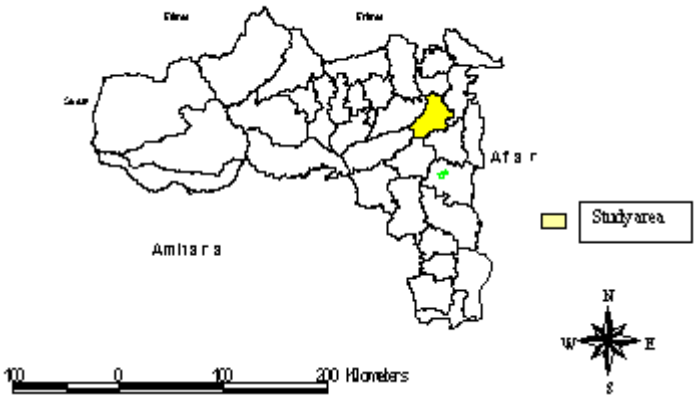


Figure 1. Location of the study in Tigray Regional State, Ethiopia

Study Animals

Abomasa of 166 local goats slaughtered in different restaurants of Mekelle town were collected for parasitological examination between November 2008 and February 2009. Goats in the area are raised with the traditional extensive system. Goats are supplied to Mekelle market from districts surrounding the town. Goats of both sexes usually aged between 1 and 4 years are slaughtered in the town.

Study design, Sampling and Sample size

The study was a cross-sectional study involving post-mortem examination of abomasa from slaughtered goats selected using a systematic random sampling technique. The sample size was determined based on 88% estimated prevalence (Kumsa and Wossene, 2006), 95% level of confidence, and 5% absolute precision (Thrusfield, 2005), requiring a minimum sample size of 162 animals. Restaurants slaughtering goats in Mekelle town were identified and those which were willing to cooperate after being briefed about the objectives of the study were included in the study.

Sample collection, processing, worm identification and count

Abomasal samples were collected from the restaurants on prearranged visits. The abomasa, after being ligated, were cut at both ends (at omasal and duodenal junctions) to separate from the rest of the digestive tract. They were transported to Mekelle Regional Veterinary Laboratory, Parasitology Department, where the laboratory examination was conducted. Standard procedures were used to recover, identify and count adult worms (MAFF, 1977; Hansen and Perry, 1994). Briefly, the abomasum was opened along the greater curvature, the contents poured into a graduated bucket, and the mucosal surface washed carefully into the bucket paying attention to the folds. The total volume was made to 2 liters from which an aliquot of 200 ml was transferred to graduated beaker while the content was stirred. The aliquot was filtered through a sieve of 250 µm apertures and the whole matter remaining on the sieve was re suspended in water. This was repeated until debries that would obscure the detection of the worms were reasonably cleared. Subsamples were placed in petridish and examined under a stereomicroscope. The numbers of worms obtained in the 200 ml aliquot were multiplied by 10 to determine the total number of worms.

Degree of infection for specific parasite species was categorized as described by Hansen and Perry (1994) as light, moderate and heavy with count of 1-500, 501-1,500, >1,500 and 1-1,000, 1,001-10,000, >10,000 for *Haemonchu*s and *T. axei*, respectively.

Data analysis

Microsoft Excel was used to store the data and in the analysis of descriptive statistics. Prevalence was calculated as a percentage of goats having at least 1 worm of a certain species of parasite out of a total of goats examined. Comparison of prevalence among months was analyzed using Chi-square test. The worm count data were log transformed [$\log_{10}$(EPG + 1)] and worm count difference between months was analyzed using one-way analysis of variance (ANOVA). STATA version 7 was used for the Chi-square and SPSS version 13 for the ANOVA.

Results

Prevalence

Two species of nematode parasites, namely *Haemonchus* spp. and *Trichostrogylus axei* were identified from the abomasa of the study goats. Out of the 166 abomasa of goats examined 126 (75.9%, 95% CI: 69.3-82.5) and 109 (65.7%, 95% CI: 58.4-73.0) were harboring *Haemonchus* spp. and *T. axei,* respectively. The overall prevalence of abomasal parasites was 91.6% (95% CI: 87.3-95.8). Eighty-three (50%, 95% CI: 35.0-65.0) of the goats had both parasites in their abomasa (Table 1).

Table 1.Prevalence of abomasal parasites in goats slaughtered in Mekelle town, northern Ethiopia (n=166)

Parasite species	No. positive	%Prevalence	95 % CI
Haemonchus spp.	126	75.9	69.3 - 82.5
T. axei	109	65.7	58.4 - 73.0
Haemonchus spp. and *T. axei*	83	50.0	35.0- 65.0
At least one parasite	152	91.6	87.3 - 95.8

Month had significant (P<0.05) effect on prevalence of both species of parasites identified (Table 2). The highest proportion of goats with at least one adult parasite was recorded in February for *Haemonchus* spp. (100%) and in December for *T. axei* (81%).

Worm burden

Mean adult parasitic worm count from abomasa of goats slaughtered in Mekelle during the study period is shown in Table 2. The recorded mean counts (considering all goats examined) were 39.5 and 55.2 for *Haemonchus* spp. and *T.axei,* respectively. The corresponding mean (±SE) adult worm count for positive goats were 52.1 (±4.4) and 84.0 (±9.8).

Significant differences (P<0.05) were observed among months in mean worm count for both *Haemonchus* spp. and *T. axei* (Table 2). Relatively highest counts were observed in February for *Haemonchus* spp. and in December for *T. axei.*

The maximum counts recorded were 270 and 600 in December for *Haemonchus* spp. and *T. axei,* respectively. *Haemonchus* spp. has shown relative highest prevalence and mean count in February.

Counts for both parasites in all positive goats observed in the study (maximum count of 270 and 600 adult worms for *Haemonchus* and *T. axei*, respectively) revealed light degree of infection.

Table 2. Monthly prevalence and worm burden of abomasal nematodes in goats slaughtered in Mekelle

Parasite species	Month	No. Examined	Prevalence				Worm burden		
			No. (%) Positive	95 % CI	χ^2	P value	Mean (±SE)	Min	Max
Haemonchus spp.	Nov	24	19(79.2)	62.9 – 95.4	11.8	0.008	30.8 (±6.4)a	0	120
	Dec	79	52(65.8)	55.4 – 76.3			29.6 (±5.3)a	0	270
	Jan	42	34(81.0)	69.1 – 92.8			37.6 (±5.2)a	0	130
	Feb	21	21(100)	-			90.5 (±13.5)b	20	220
	Overall	166	126(75.9)	69.3-82.5			39.5 (±3.8)	0	270
T. axei	Nov	24	5(20.8)	4.6 – 37.1	33.3	0.000	9.6 (±4.4)a	0	80
	Dec	79	64(81.0)	72.4 – 89.7			86.7 (±13.6)b	0	600
	Jan	42	30(71.4)	57.8 – 85.1			39.5 (±6.3)b,c	0	140
	Feb	21	10(47.6)	26.3 – 69.0			26.8 (±5.9)a,c	0	80
	Overall	166	109(65.7)	58.4-73.0			55.2 (±7.1)	0	600

a,b,cMeans followed by different superscripts differ significantly (P<0.05)

Discussion

Only two genera of nematodes namely *Haemonchus* and *Trichostrogylus* were detected in the abomasa of the study goats. This is in agreement with the reports of similar studies in the northern and eastern parts of the country (Abebe and Esayas, 2001; Kumsa and Wossene, 2006; Menkir *et al*., 2007; Teshale and

Aragaw, 2014). However, studies in southern Ethiopia (Asha and Wossene, 2007; Thomas *et al*., 2007; Bitew *et al*., 2011; Demissie *et al*., 2013) frequently documented the occurrence of another abomasal nematode parasite namely *Teladorsagia* (*Ostertagia*) spp. in addition to the two genera recovered in this study. *Ostertagia* is known to be especially important in temperate climates and in subtropical regions with winter rainfall (Urquhart *et al*., 1996).

The high overall prevalence (91.6%) of abomasal nematodes in goats in Mekelle, revealed by the current study is comparable to 95% (Abebe and Esayas, 2001) and 90.2% (Kumsa and Wossene, 2006) prevalence reported from eastern and 87.1% from southern Ethiopia (Thomas et al., 2007). It was, however, higher compared to 45.2% (Teshale and Aragaw, 2014) and 61.8% (Bitew et al., 2011) prevalence reported from northwestern and southern Ethiopia, respectively. Such high prevalence of abomasal helminth parasites in goats suggests existence of favorable environmental conditions for survival and development of nematode larvae to infective stage in the study area. It also suggests that goats, which are normally browsers, may have been forced to graze perhaps due to lack of adequate browsing material in the study area (Le Jambre and Royal, 1976).

The recorded high prevalence of *Haemonchus* spp. (75.9%) in our study was in agreement with 76.5% (Thomas *et al*., 2007) and 75.2% (Demissie *et al*., 2013) prevalence reported from southern Ethiopia. Bitew *et al*. (2011) from southern Ethiopia (55.9%) and Teshale and Aragaw (2014) from northwestern Ethiopia (43.5%) have reported much lower prevalence. Other works however recorded higher prevalence of the parasite in slaughtered goats in Ethiopia (Abebe and Esayas, 2001; Kumsa and Wossene, 2006).

Comparable prevalence of *T. axei* (64.3%), to our study (65.7%), was recorded in eastern Ethiopia by Abebe and Esayas (2001). However, several other studies from different parts of the country recorded lower prevalence of the parasite in slaughtered goats (Kumsa and Wossene, 2006; Thomas *et al*., 2007; Bitew *et al*., 2011; Demissie *et al*., 2013; Teshale and Aragaw, 2014). These differences in prevalence of the parasites among the studies are most likely due to difference in environmental conditions, as they affect the development and survival of the parasites outside and inside the host (Urquhart *et al*., 1996).

The average worm count observed for both *Haemonchus* spp. (39.5) and *Trichostrongylus axei* (55.2) in our study were much lower compared to reports of sev-

eral similar earlier studies in Ethiopia (Abebe and Esayas, 2001; Menkir *et al*., 2007; Thomas *et al*., 2007; Teshale and Aragaw, 2014). This may be explained by difference in agroclimatic conditions among the study areas which affect favorability of the environment for development and survival of the free living stages of the parasites (Urquhart *et al*., 1996). It may also be partly explained by difference in season of study among the reports. Our study was conducted during the dry season where parasite prevalence and burden are usually low (Bekele *et al*., 1987; Tembely *et al*., 1997; Menkir *et al*., 2007).

In the present study all of the goats harboring adult abomasal parasites were affected by light degree of infection based on the interpretation suggested by Hansen and Perry (1990). In a somewhat different result Kumsa and Wossene (2006) in goats from eastern Ethiopia found a few animals with heavy infection, while majority of the infected goats were with light to moderate degree of infection. The reasons for light degree of infection for both parasites notwithstanding high prevalence need further investigation.

Monthly prevalence and total adult worm burden of the identified abomasal parasites in the study period had shown difference which was relatively highest in February for *Haemonchus* spp. and in December for *T. axei*, although there was no change in degree of infection. This difference is difficult to explain as there was no significant rain during the study period in the area. Under Ethiopian highland conditions, moisture appears to have the most important effect on the development and survival of eggs and larvae of nematodes (Haile *et al*., 2010).

Conclusions

The present study demonstrated high prevalence of pathologically the most important nematode parasite of ruminants- *Haemonchus* spp.- along with *T. axei* in goats in the study area, albeit with light degree of infection. The results suggest insidious losses of production in goats in the study area due to helminth parasites.

Acknowledgements

The authors would like to thank Dr. Girmay W/Selassie, head of Mekelle Regional Veterinary Laboratory for his unreserved help in facilitating the study. Owners of Mekelle restaurants with their butcher men are also appreciated for

their kindness in provision of samples. Traders of goats in Mekelle, especially Ato Nigus, are gratefully acknowledged for provision of information.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abebe, W. and Esayas, G., 2001. Survey of ovine and caprine intestinal helminthosis in eastern part of Ethiopia during the dry season of the year. *Rev. Med. Vet*., 152, 379–384.

Aga, S., Tolossa, Y. and Terefe, G., 2013. Epidemiology of gastrointestinal nematodes of Horro sheep in Western Oromia, Ethiopia. J. Vet. Med. Anim. Health. 5 (10), 296–304.

Allonby, E.W. and Urquhart, G.M., 1975. The epidemiology and pathogenic significance of haemonchosis in a merino flock in east Africa. Vet. Parasitol. 1, 129–143.

Asha, A. and Wossene, A., 2007. Gastrointestinal tract nematodosis of small ruminants in three different agro-ecological zones in southern Ethiopia. Ethiop. Vet. J., 11(1), 83–94.

Asmare, A., Sheferaw, D., Aragaw, K., Abera, M., Sibhat, B., Haile, A., Kiara, H., Szonyi, B., Skjerve, E. and Wieland, B., 2016. Gastrointestinal nematode infection in small ruminants in Ethiopia: A systematic review and meta-analysis. *Acta Trop*., 160, 68-77.

Bekele, T., Mukasa-Mugerwa, E. and Scholtens R.G., 1987. Seasonal changes in nematode faecal egg counts of sheep in Ethiopia. International Livestock Centre for Africa (ILCA). Bulletin No. 29.

Bitew, M., Yeshitla, A. and Kidist, B., 2011. Abomasal and small intestinal nematodes ruminants slaughtered in different restaurants in Hawassa. *Vet. Res*., 4 (2), 39–44.

Chaudary, R. F. Khan, U. F. M., and Qayyum, M., 2007. Prevalence of *Haemonchus contortus* in naturally infected small ruminants grazing in the Potohar area of Pakistan. *Pakistan. Vet. J*., 27(2), 73-79.

CSA (Central Statistical Agency), 2017. Report on livestock and livestock characteristics (private peasant holdings). Agricultural sample survey 2016/17[2009 e.c.], Volume II Statistical bulletin 585.

Demissie, T., Tesfaye, D., Fekadu, A. and Asefa, I., 2013. Study on abomasal nematodes of sheep and goats: comparison and characterization of vulvar morphology of Haemonchus in Hawassa, Ethiopia. Afr. J. Agric. Res., 8 (39), 4922–4927.

FAO (Food and Agriculture Organization of the United Nations), 2004. Livestock sector brief: Ethiopia. Livestock information, sector analysis and policy branch (AGAL), FAO, Rome, Italy.

Githigia, S.M., Thamsborg, S.M., Munyua, W.K. and Maingi, N. (2001). Impact of gastrointestinal helminths on production in goats in Kenya. Small Rumin. Res., 42, 21-29.

Haile, A., Gashaw, A., Tolamariam, T., and Tibbo, M., 2010. Epidemiology of nematode parasites of sheep around Jimma, South Western Ethiopia. Trop. Anim. Hlth. Prod., 42(5), 825–831.

Haile, A., Hassen, H, Gatew, H., Getachew, T., Lobo, R. N. B. and Rischkowsky, B., 2018. Investigations into nematode parasites of goats in pastoral and crop livestock systems of Ethiopia. Trop. Anim. Hlth. Prod., 50(3), 643-650.

Hansen, J. and Perry, B., 1994. The Epidemiology and Control of Helminth Parasites of Ruminants, A hand book, International Laboratory for Research on Animal Disease. Nairobi, Kenya. Pp 3-149.

Kebede, T., Haile, A. and Dadi, H., 2012. Smallholder goat breeding and flock management practicesin the central rift valley of Ethiopia. Trop. Anim. Hlth. Prod., 44, 999–1006.

Kumsa, B. and Wossene, A., 2006. Abomasal nematodes of small ruminants of Ogaden region, eastern Ethiopia: Prevalence, Worm burden and species composition. Rev. Med. Vet., 157(12), 27-32.

Legesse, G., Siegmund-Schultze, M., Abebe, G., Valle Zárate, A., 2010. Economic performance of small ruminants in mixed-farming systems of southern Ethiopia. Trop. Anim. Hlth, Prod., 42, 1531–1539.

Le Jambre, L.F. and Royal, W.M., 1976. A comparison of worm burden in grazing Merino sheep and Angora goats. Aust. Vet. J., 52, 181–183.

MAFF (1997): Manual of Veterinary Parasitological Laboratory Techniques. Tech. Bull. No., 18: Her majesty's Stationary office, London 1-5.

MCPPP (2007): Mekelle town Municipality. Mekelle City Plan Preparation Project.

Menkir, M., Uggla, A., Waller, P.J., 2007. Prevalence and seasonal incidence of nematode parasites and fluke infections of sheep and goats in eastern Ethiopia. *Trop. Anim. Hlth. Prod*., 39, 521–531.

Over, H. J., Jansen, J. and van Olm, P. W., 1992. Distribution and impact of helminth diseases of livestock in developing countries. FAO Animal Production and Health Paper 96.

TBOANR, 1999. Tigrai Bureau of Agriculture and Natural Resources, Annual Report.

Tembely, S., Lahlou-kassi, A., Rege, J.E.O., Sovani, S., Diedhiou, M.L., Baker, R.L., 1997. The epidemiology of nematode infections in sheep in a cool tropical environment. *Vet. Parasitol.*, 70, 129–141.

Teshale, A. and Aragaw, K., 2014. Abomasal nematodes of small ruminants slaughtered in Bahir Dar town, Northwest Ethiopia. *J. Vet. Med. Anim. Health*, 6 (10), 257–262.

Thomas, N., Teshale, S., and Bersissa, K. (2007): Abomasal Nematodes of Sheep and Goats Slaughtered in Awassa (Ethiopia): Species Composition, Prevalence and Vulvar Morphology. *Helminthologia*, 44, 70-75.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd ed. Blackwell science Ltd, London, pp. 228-246.

Tolera, A. and Abebe, A., 2007. Livestock production in pastoral and agro-pastoral production systems of southern Ethiopia. *Livestock Res. Rural Dev.*, 19(12), 1–13.

Torres-Acosta, J.F.J. and Hoste, H., 2008. Alternative or improved methods to limit gastro-intestinal parasitism in grazing sheep and goats. *Small Rumin. Res.*, 77, 159–173.

Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M., and Jennings, F. W. (1996): Veterinary Parasitology. 2nd Edition, Black Well Science. PP 3 - 26.

Woldemariam, D.L., 2005. Nematode prevalence, helminth management practices and anthelmintic resistance in small ruminants in the mid-rift valley of Ethiopia. Doctor of Veterinary Science Thesis, University of Pretoria, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Republic of South Africa.

Short communication

Important role of Non Tuberculous Mycobacteria in Non-Human primate TB testing in Ethiopia

Rea Tschopp [1,2,3] and Liya Wassie[1]*

[1] Armauer Hansen Research Institute (AHRI), PO Box 1005, Jimma Road, Addis Ababa, Ethiopia

[2] Swiss TPH, Socinstr 57, 4002 Basel, Switzerland

[3] University of Basel, Basel, Switzerland

** Corresponding author: Rea Tschopp, rea.tschopp@swisstph.ch*

Abstract

Tuberculosis is an important disease in captive non-human primates (NHP) but remains challenging to diagnose accurately. The tuberculin skin test (TST) remains the standard test used worldwide, whereby the single intradermal tuberculin test (SIDT) in the eye-lid is the most frequently used method to diagnose TB in NHP. In this study, 9 NHP (5 baboon *spp*.) and 4 geladas (*T. gelada*) were tested for TB using the Primagam (whole blood immunoassay) in order to assess the feasibility of relocation into the wild. Although the results showed that all species reacted to non-tuberculous mycobacteria (NTM), the geladas showed a consistent distinct reaction pattern with very high reactivity to NTM. The results suggest that using the SIDT in Ethiopian primates, particularly the endemic geladas, would lead to a high number of false positive animals, highlighting the current challenge to accurately diagnose NHP for TB, which could help in the conservation efforts in the country.

Key words: Ethiopia; TB; Non-human primate; Gelada; non-tuberculous mycobacteria

Introduction

Tuberculosis (TB), although not occurring naturally in wild non-human primate (NHP) populations, is a major health risk for animals that have close contact to people and/or that consume meat from TB infected animals (Keet *et al*., 2000, Montali *et al*., 2001, Martino *et al* 2006). *Mycobacterium tuberculosis* (*M. tuberculosis*) and to a much lesser extent *M. bovis* are the two main patho-

gens causing TB in NHP (Kaufman and Anderson 1978). TB, besides being a public health threat, is a highly contagious and costly disease in captive NHP in terms of cost of animal losses, cost of diagnostics, and cost of treatment for exposed staff. It requires regular and accurate screening to avoid outbreaks in these populations. Reactors are usually eliminated in order to prevent further disease spread. The choice of diagnostic test is hence of importance, in order to avoid false negative animals that can continue to spread the disease and false positive animals that would be sacrificed unnecessarily. The test should also ideally be able to detect the disease at an early stage as well as detect latent cases. Like in humans, TB diagnostic however, remains a challenge in NHP. There is currently not a single test that meets all requirements on sensitivity, specificity, user friendliness, low cost and available logistics.

The tuberculin skin testing is a standard diagnostic tool to screen NHP for TB in captive animals (Bushmitz *et al*., 2009). The eyelid test is usually preferred as compared to abdominal testing, where purified protein derivative (PPD) is injected intradermally into the eyelid under general anesthesia and reaction (grade of swelling/drooping) observed and interpreted after 72 hours, under a second anesthesia. The test is based on a delayed hyper sensitivity response against a mycobacterial antigen (delayed-type hypersensitivity) (Garcia *et al.,* 2004; Lerche *et al*., 2008; Lin *et al*., 2008). Blood tests such as *in-vitro* immune stimulation assay are also used to a lesser extent. They are quantifiable, require general anesthesia only once, have good sensitivity and specificity and can detect TB at an early stage (Garcia *et al*., 2004).

Material and Methods

In this small study conducted in 2016 in Ethiopia, 9 captive NHP from 2 different facilities in Addis Ababa were screened for TB. The main objective for screening was to assess the possibility of releasing individuals into the wild. Four olive baboons (*P. Anubis*), 1 Hamadryas baboon (*P. hamadryas*) and 4 Geladas (*T. gelada*) were included in the study. Two were juveniles, 3 were sub-adults and 4 were adults. Six out of 9 were males (67%). Due to a shortage of PPD and anesthetics, a blood test was solely performed on all NHP, hence requiring only one anesthesia. All procedures were performed by a trained professional wildlife veterinarian, assuring that welfare standard for animals were met. Volume of drawn venous blood was dependent on the size of the animal (maximum 2 ml), drawn into heparinized tubes and transported to the TB lab at the Armauer Hansen Research Institute (AHRI), Addis Ababa within 2

hours. The commercial PRIMAGAM-primate IFG test (Prionics AG, Schlieren, Switzerland) test was used. a The test procedure followed as indicated by the manufacturer. The PRIMAGAM is derived from the classical BOVIGAM test used in cattle. The test is a rapid *in-vitro* lymphocyte stimulation assay, where lymphocytes are presented to antigens in whole blood culture. It detects reactivity to tuberculin antigens by measuring the IFN-γ produced by lymphocytes that had previous contact with the pathogens using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). It has relatively good sensitivity (68%) and excellent specificity (97%) as compared to the TST (Garcia *et al*., 2004). The tests were run in duplicates. Optical density (OD) was measured for bovine and avium PPD. The test results were interpreted as positive for TB if the OD of PPD-B – PPD-A > 0.05 OD units.

Results

The tests results are shown in Fig. 1. Following the definition cut-off of the manufacturer, none of the 9 tested animals were positive for TB. Five out of nine (55%) animals had positive reactions to bovine PPD. These animals would have most likely reacted positively in the eye lid test. However, taking into account reactions to avium PPD, the final diagnosis was negative for TB in all cases. Gelada monkeys (individuals 6, 7, 8 and 9) showed a consistent distinct pattern of *M. avium* PPD reactions by being more elevated than the reaction with bovine PPD, which were consistently high as well.

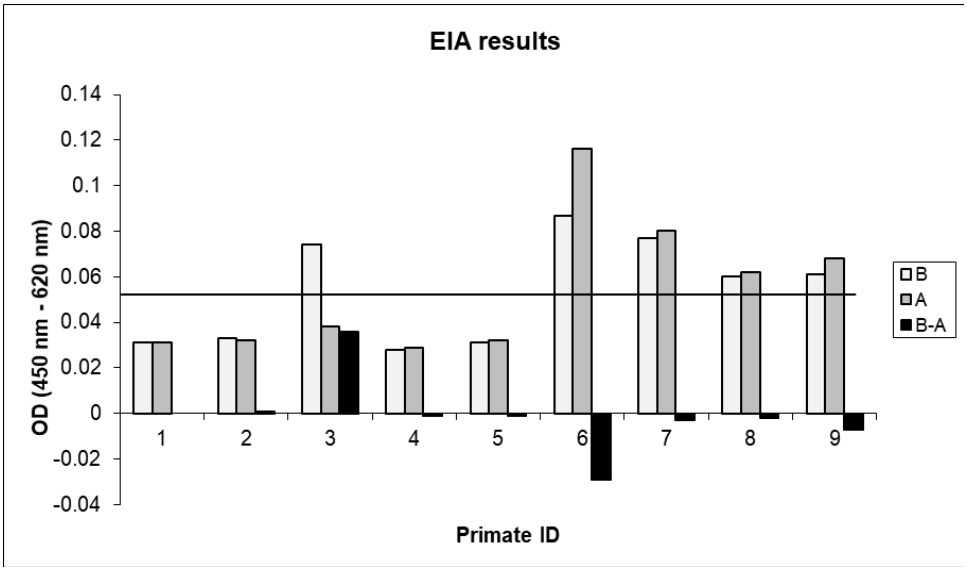


Fig 1. PRIMAGAM results of 9 captive non-human primates. B= OD PPD bovine; A= OD PPD avium; B-A = difference OD PPD bovine and OD PPD avium. Cut-off for TB positivity set at B-A > OD=0.05 (shown by a horizontal line).

Discussion

To our knowledge this is the first published information on TB testing in wild caught gelada monkeys. Geladas are a species of old-world monkeys, endemic to the Ethiopian Highlands. They are terrestrial, gregarious and the only graminivorous primates (true grazers). They spend their days foraging on grass and herbs, hence being constantly close to the ground. This feeding behavior is very likely an explanation for the high exposure to environmental mycobacteria as compared to the baboon species.

In Ethiopia, the non-tuberculosis mycobacterial burden has been shown to be high in humans (Mathewos *et al.,* 2010, Workalemahu *et al.,* 2011, Gumi *et al*., 2012), livestock (Berg *et al.,* 2009) and wildlife species (Tschopp *et al.,* 2010). The single intradermal skin test (eye-lid test), as used per recommendation of the European Primate Veterinary Association Working Group is in the Ethiopian context is thus not advisable. Over half of the NHP would have been false positive reactors in this study. Comparative skin testing is hence mandatory.

Also, a high number of anergic cases have been observed in old world monkeys, while using the TST, particularly in baboons (Montali *et al*., 2001). Although highly specific, and able to detect early stages and latency of TB, the IFN-γ test is known to have some pitfalls as well leading to false negative reaction (Garcia *et al.,* 2004). Hence, for both the skin test and blood test, results are affected by the primate species tested. For instance, *Cynomolgus macaques* have been described as having a lot of false negative results with the TST as with the primagam (Garcia *et al.,* 2014). Since both tests fail to 100% identify TB positive animals, a combination of different tests would increase the likelihood of detecting these positive reactors. In addition, species specific diagnostic approaches should be taken in account, particularly with baboon spp and geladas, particularly in the context of the conservation of these species. However, in a poor resource country like Ethiopia, combination diagnostics could prove challenging (e.g. lack of wildlife veterinary expertise, reagent and lab availability, costs of diagnostics).

Acknowledgements

The authors are thankful to the staff of AHRI for their logistic and financial support, to EWCA and to the Born Free Foundation for purchasing the reagents.

References

Berg, S., Firdessa, R., Habtamu, M., Gadisa, E., Mengistu, A., Yamuah, L., et al. 2009. The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. *PLoS ONE* 4(4): e5068. https://doi.org/10.1371/journal.pone.0005068

Bushmitz, M., Lecu, A., Verreck, F., Preussing, E., Rensing, S., et al. 2008. Guidelines for the prevention and control of tuberculosis in non-human primates: recommendations of the European Primate Veterinary Association Working Group on tuberculosis. *J. Med. Primatol*., 38(1), 59-69. https://doi.org/10.1111/j.1600-0684.2008.00303.x.

Garcia, M.A., Yee, J.A.L., Bouley, D.M., Moorhead, R. and Lerche, N.W. 2004. Diagnosis of Tuberculosis in Macaques, Using Whole-Blood In Vitro Interferon-Gamma (PRIMAGAM) Testing. *Comp. Med*., 54(1), 86-92.

Gumi, B., Schelling, E., Berg, S. et al. 2012. Zoonotic Transmission of Tuberculosis Between Pastoralists and Their Livestock in South-East Ethiopia. *EcoHealth*, 9 (2), 139-149. Doi:10.1007/s10393-012-0754-x.

Kaufmann, A.F., and Anderson, D.C. 1978. Tuberculosis control in nonhuman primate colonies, p227-234. In R.J, Montali (ed) Mycobacterial infections in zoo animals. Smithsonian Institution Press, Washington DC.

Keet, D.F., Kriek, N.P.J., Bengis, R.G., Grobler, D.G., and Michel, A. 2000. The rise and fall of tuberculosis in a free-ranging chacma baboon troop. *Onderstepoort J. Vet. Res.*, 67(2),115-122.

Lerche, N.W., Yee, J.A.L., Capuano, S.V., and Flynn, J.L. 2008. New Approaches to Tuberculosis Surveillance in Nonhuman Primates. *ILAR* J., 49(2), 170-178. https://doi.org/10.1093/ilar.49.2.170.

Lin, P.L., Rodgers, M., Smith, L., Bigbee, M., Myers, A., Bigbee, C., Chiosea, I., Capuano, S.V., Fuhrman, C., Klein, E., and Flynn, J.A.L. 2009. Quantitative Comparison of Active and Latent Tuberculosis in the Cynomolgus Macaque Model. *Infect. Immun.*, 77(10), 4631-4642.

Mathewos, B., Kebede, N., Kassa, T., Mihret, A., and Getahun, M. 2015. Characterization of mycobacterium isolates from pulmonary tuberculosis suspected cases visiting

Tuberculosis Reference Laboratory at Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia: a cross-sectional study. *Asian Pac. J. Trop. Med.*, 8(1), 35-40. doi: 10.1016/S1995-7645(14)60184-X.

Montali, R.J., Mikota, S.K., and Cheng, L.I. 2001. *Mycobacterium tuberculosis* in zoo and wildlife species. *Rev Sci Tech.*, 20(1), 291-303. http://dx.doi.org/10.20506/rst.20.1.1268.

Martino, M., Hubbard, G.B. and Schlabritz-Loutsevitch, N. 2006. Tuberculosis (*Mycobacterium tuberculosis*) in a pregnant baboon (*Papio cynocephalus*). *J. Med. Primatol.*, 36 (2).108–112. doi:10.1111/j.1600-0684.2006.00166.x

Tschopp, R., Berg, S., Argaw, K., Gadissa, E., Habtamu, M., Schelling. E., Young, D., Aseffa, A., and Zinsstag, J. (2010). Bovine tuberculosis in Ethiopian wildlife. *J. Wildl. Dis.*, 46(3). 753-762. https://doi.org/10.7589/0090-3558-46.3.753

Workalemahu, B., Berg, S., Tsegaye, W., Abdissa, A., et al. 2013. Genotype diversity of mycobacterium isolates from children in Jimma, Ethiopia. *BMC Res. Notes*, 6, 352.

Ethiopian Veterinary Journal

Guidelines for Authors



2019

Ethiop. Vet. J. is the Official Scientific Organ of the Ethiopian Veterinary Association

Ethiopian Veterinary Journal (*Ethiop. Vet. J.*)

Objectives and Scope

The Ethiopian Veterinary Journal (*Ethiop. Vet. J.*) is a multidisciplinary peer-reviewed journal intended to promote animal health and production of national and regional/international importance. The journal publishes review articles, original research articles, short communication as well as technical notes in English. Under special circumstances, articles in Amharic may be considered for publication.

Ethiop. Vet. J., is published by the Ethiopian Veterinary Association (EVA)

Copyright © Ethiopian Veterinary Association (EVA)
Ethiop. Vet. J.,
ISSN: 1683-6324

All articles as well as the editorials published in the Ethiopian Veterinary Journal represent the opinion of the author(s) and do not necessarily reflect the official view of the Ethiopian Veterinary Association, the Editorial Board or the institution within which the author(s) is/are affiliated unless this is clearly stated. Furthermore, the author(s) is/are fully responsible for the contents of the manuscript and for any claim or disclaim therein.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the Association.



Ethiop. Vet. J., is the Official Scientific Organ of the Ethiopian Veterinary Association

ETHIOPIAN VETERINARY JOURNAL

(*Ethiop. Vet. J.*)

Objectives

- To stand as the official scientific organ of the Ethiopian Veterinary Association and serve as medium of communication with professionals in the fields of animal health and production and other related disciplines.
- To effectively disseminate research outputs in all spheres of veterinary science, veterinary public health, animal production and other related fields of study.
- To help promote agricultural development in Ethiopia and the subregion as a whole through generation via research of technologies, methods etc and dissemination of scientific knowledge to stakeholders and ultimately to end users.

Type of papers

- Original research papers
- Review articles
- Short communications

Original articles

This includes original articles presenting outputs from basic, applied and adaptive research activities related to animal and public health and diverse aspects of animal production in Ethiopia, and from regional and international sources. The material must not have been previously submitted or published elsewhere. The research article should not contain more than 6000 words.

Review articles

These include papers covering baseline data/information as well as advances in the field of veterinary medicine, public health and related fields of research and development. Often review articles will be prepared by specialist or researchers actively engaged in particular area of study for a considerable period of time with proven success. This must be supported by a strong track record

of publications in the area of the proposed review. Before submitting a review, authors must first contact the Editor with an outline of a proposed review. Acquisition of such papers my also be done through invitation. The review article should not contain more than 9000 words

Short communications

This is devoted to entertain results of original research, selected clinical case reports, brief scientific notes including preliminary results, scientific observation, experimental techniques, recent technological advances and news of interest in the field. Short communication must be written in a synthesis form where all necessary information are presented in a condensed manner. The material must not have been previously published elsewhere. Short communication should not contain more than 3000 words

Guidelines for authors

The Ethiopian Veterinary Journal (*Ethiop. Vet. J.*) is a multi-disciplinary and peer-reviewed publication intended to promote animal health and production of national, regional and international importance. All submitted manuscripts should be in English.

Submissions

Electronic copy in Word file should be addressed to the Editorial-Office. All manuscripts should be accompanied with a letter (or a filled Manuscript Submission and Copyright Transfer Form) signed by all authors, who clearly state that the paper, other than oral presentation or abstract, has not been submitted for publication elsewhere in any form. The Editorial Board reserves the right to accept or reject any paper submitted.

Manuscripts

In general, all submitted manuscripts must conform to the requirements set by the Journal. Failure to follow instruction in the preparation of manuscripts may result in total rejection or delay in publication. It should be prepared with Times New Roman 12 pt, double-spaced, minimum 1.5 cm margins. To facili-

tate the review process give line numbers in Arabic numerals. Give also page number in the lower middle of each page, starting from the title page.

Title page

The title page should consist of:

a) Title of the article, which should be concise and descriptive enough; should be in sentence case, and justified left; avoid abbreviations;
b) Name(s) of author (s), institutional affiliation, email addresses of all authors in italics (s); and
c) Name and address of the corresponding author, in case of more than one authors.

Abstract

The second page should provide an abstract of not more than 300 words summarizing the background, objective, materials and methods, major findings and their significance, and conclusions. Avoid the use of undefined abbreviations.

Keywords

Below the abstract, in a separate line, keywords, up to six in number should be given in alphabetical order separated by ";".

Introduction

Give sufficient information on the associated background zooming in to the subject in question; articulate the specific research question, and the contribution of your current research in bridging the gap, followed by statement of objective(s) is in order.

Materials and methods

Under this section, briefly state the materials used, the selection of study animals, subjects, herds or materials, and the design for observational or experimental study and the nature of controls. Briefly describe the methods and procedures followed in sufficient detail and give reference; if new or modified methods are used, give justification for using them. Precisely specify apparatus, instrument or chemicals used in terms of manufacturer's name and ad-

dress in parentheses. Similarly, drugs, biologicals, medicinal plants or others used should be sufficiently described in terms of generic name(s) or scientifically accepted identifications, dose(s), and route(s) of administration. Describe sufficiently the statistical methods used and how the magnitude or precision of analysis results reached.

Results

Quantify qualitative findings and present them with appropriate indicators of measurement of error or uncertainty, such as by using confidence intervals. Likewise, when reporting quantitative data, authors must indicate the extent of variability by either using standard deviation or standard error. Present the results in logical sequence in the text, tables, or illustrations. The results have to be presented in the same order as the questions raised in the objective(s) and methods sections. Results should be concise and no need of interpretation. In clinical or therapeutic trials, report complications or losses or even dropouts of such observations giving numbers. Support all findings by using appropriate statistical analysis.

Tables should be complete enough to be informative. They should be presented in separate pages, numbered consecutively using Arabic numerals and provided with captions. Avoid using vertical lines to separate columns in Tables. Details essential to further explain specific aspects of the Table should be given as a footnote below the table, by using appropriate symbols or lower case letters as superscripts.

Figures and the captions should also be placed in separate sheets, numbered in Arabic numerals according to the sequence of their appearance in the text. Lengthy and complicated Tables and Figures are discouraged. When data are reported, avoid also duplications in presenting them. In other words, the same data should either be presented in a Table or Figure or text but not more than one of these options. All Tables and illustrations must be cited in the text. Photographs of only good quality with TIFF, PDF, JPEG format with minimum dpi of 300 are accepted. Colored illustrations are considered if the costs are covered by the author.

Discussion

Under this heading you should explain the findings and the associated interpretations. Relate the results with the objective of the study and with previ-

ously published work. Here emphasize the new and important aspects of the study and not repeat the results. In the discussion, clearly indicate the significance of the work and implications of the findings for future research.

Conclusion

This section should be separately presented with supporting evidences based on the major findings of the study. Appropriate recommendations can be made if necessary

Acknowledgements

Acknowledgments should be briefly stated after the Conclusions. Under this, technical, financial and material support can be mentioned.

Conflict of interest

Authors should declare that they have no conflict of interest.

References

All citations of publications in the Text, Tables and illustrations must follow the requirements. Show such references by author's name (without initials) followed by year of publication in Arabic numerals and all that in parentheses. When reference is made to a publication written by more than two authors, then indicate the first author's name (without initials) followed by "*et al*.,". When you want to use name of author out of paranthesis in case of more than two authors use last name of first author *et al* (year). More than one references cited together should follow a chronological order and separated by ";" in a parenthesis. Personal communications and unpublished work must not be included in the reference list. They should be mentioned in the text only. Examples: (Dagnachew, 2004, personal communication). (Gopelo, 2004, unpublished).

All cited publications must be presented in the list of references. In such a list, the authors' names are presented in alphabetical order and then chronologically by author. All publications by the single author precede those multi-authored publications. Works of the same author should be arranged according to publication dates. Publications by the same author(s) with the same year should be listed, using 'a', 'b', 'c', etc after the year, eg. 2004a, 2004b, etc. If

the number of authors is more than six, '*et al.,*' should be used after the sixth author. Titles of references should be given in the original language, except for instances involving non-Latin alphabets, in which case the title should be transliterated and a notation identifying the language, for example 'in Amharic', be added. Abbreviations of the titles of periodicals mentioned in the reference list must be according to the International List of Periodical Title Word abbreviations.
Use the following method for presenting references:

A) Article in journals:

Ameni, G., Miorner, H., Roger, F. and Tibbo, M., 2000. Comparison between comparative tuberculin and gamma-interferon tests for the diagnosis of bovine tuberculosis in Ethiopia. *Trop. Anim. Hlth. Prod*., 32, 267-267.

B) Accepted Articles in press with doi.no.

Jobre, Y., Malone, J. B., McCarroll, J. C., Erko, B., Mukaratirwa, S. and Xinyu, Z., 2001. Satellite climatology and the environmental risk of Schistosoma mansoni in Ethiopia and east Africa. *Acta Trop*., (*In press*).

C) Papers in proceedings

Tegegne, A., Wirtu, G., Mukasa-Mugerwa, E. and Kassa, T., 1994. Oestrus phenomenon and oestrus detection efficiency using androgenized cows and entire bulls in Boran and Boran x Friesian crossbred cows. In: Proceedings, Advances in Tropical Agriculture in the 20th Century and Prospectus for the 21st: TA 2000, 4-9 September 1994, Port-of- Spain, Trinidad.

D) Books or chapters in books:

Thrusfield, M., 1995. Veterinary Epidemiology, 2nd edition, Blackwell Science Ltd, Oxford, UK.

Malone, J. B and Jobre, Y., 1999. Predicting outbreaks of fasciolosis from Ollernshaw to satellites. In: Dalton, J.P. (Ed.), Fasciolosis. CBA International Publications, Cambridge, Pp. 151-183.

E) Organizations as author:

OIE, 1992. Bovine tuberculosis. OIE manual for diagnostic techniques of livestock diseases. Office International des Epizooties (OIE), Paris, France. Pp. 287-296.

Nomenclature

Authors, reviewers as well as editors should follow the rules governing biological nomenclature, as indicated in the International Code of Botanical Nomenclature, the International Code of Nomenclature of Bacteria, the International Code of Zoological Nomenclature and the Standardized Nomenclature of animal Parasitic Diseases. All biotica (crops, plants, insects, birds, mammals, etc.), with the exception of common domestic animals, must be identified by scientific names when such terms are used first.

All biocides and other organic compounds should be identified by their Generic names when first used in the text; likewise, the active ingredients of all formulations should be identified. For chemical nomenclature, the conventions of the International Union of Pure and Applied Chemistry and the official recommendations of the IUPAC-IUB Combined Commission on Biochemical Nomenclature should be observed.

Ethics

When reporting experiments involving animals, authors are expected to have observed all ethical standards on the care and use of animals or any pertinent national law. The Editorial Board reserves the right to reject papers that have been judged to have subjected animals to unnecessary handling or exposure to unacceptable pain or detention. Experimental studies should be accompanied by institutional ethical clearance.

Units of measurement

All measurements should be reported in SI units; examples are meter, kilogram, liter and degree Celsius. All dates in manuscripts should be based on the Gregorian Calendar. When reporting financial matters on data collected within Ethiopia, the preferred currency to use is Birr, with exchange rates indicated in US Dollar.

Abbreviations

Use standard abbreviations only. The full terms for which an abbreviation stands should precede its first use in the text. Abbreviations of the titles of periodicals mentioned in the reference list must be according to the International List of Periodical Title Word Abbreviations. Such a list can be avail-

able from the Editorial Office upon request. E.g 'Veterinary Pathology' as *'Vet. Pathol.,*', 'Veterinary Parasitology' as *'Vet. Parasitol.,*', 'Tropical Animal Health and Production' as *'Trop. Anim. Hlth. Prod.,*', 'Preventive Veterinary Medicine' as *'Prev. Vet. Med.,*', 'Revue Medecine Veterinaire' as *'Revue Med. Vet.,*', 'Acta Tropica' as *'Acta Trop.,*', 'Journal of Helminthology' as *'J. Helminthol.,*' 'East African Medical Journal' as *'E. Afr. Med. J.,*', etc…
NB: All journal citations in reference list must be written in ***ITALICS.***

Copyright

Authors are expected to observe all copyright related matters. It has to be noted that opinions expressed by the author(s) are not necessarily the views of the Journal or the Association. Authors assume full responsibility for the contents of the manuscript and for any claim or disclaim therein. All submissions are also with the understanding, knowledge and consent of copyright transfer to the Association.

Proofs and reprints

Proofs will be sent to the corresponding author. They should be corrected and returned within 48 hrs. If this is not done in the specified period, for timely publication of the Journal, the editorial staff will have it proofed and published without the author's correction.

Mailing address

All contributions must be addressed to:

The Editorial Office
Ethiopian Veterinary Journal (Ethiop. Vet. J.) by email.
Email: evjeditorialoffice@gmail.com

Manuscript submission and copyright transfer form

All EVA publication submissions require completion of the following form addressed to:

Ethiopian Veterinary Journal
P.O. Box 2462, Addis Ababa, Ethiopia
Tel. 251-118697868
Email: evjeditorialoffice@gmail.com

1. Manuscript title: ----------

---
2. Name of author(s): ----------

---
3. Corresponding author:
Name: ----------

Institution: ----------

P.O. Box: ----------

City/Town: ----------

Telephone: ----------

Fax: ----------

Email: ----------
---
4. The above indicated manuscript is submitted to appear in: (check only one)
_____ The proceedings
_____ The journal (*Ethiop. Vet. J.*)
5. If the manuscript is submitted to the Journal, the column proposed is: (check only one)
_____ Original articles
_____ Review and feature articles
_____ Short communications, clinical/case Reports
6. The author(s) hereby:

6.1. Agree to assume full responsibility for the contents of the manuscript and for any claim or disclaim therein.

6.2. If the submission is to the Journal, that no similar paper other than oral presentation or the abstract has been or will be submitted for publication elsewhere prior to the written decision of the Editor-in-Chief.

6.3. Agree to the transfer of the copyright to the Association.

Name _____ Signature_____

Date_____