Ethiopian Veterinary Journal

Year: 2019 Volume: 23, No. 1



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 (1)
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

Carcass characteristics of draught cattle released for beef in Eastern Ethiopia
Ewonetu Kebede Senbeta and Ashenafi Getachew Megersa.....*1*
https://dx.doi.org/10.4314/evj.v23i1.1

Seroprevalence of bovine leukemia virus infection in contrasting farming systems in Kenya
Kathambi E. K., Gitau G. K., Muchemi G. M., VanLeeuwen J.and Kairu-Wanyoike S.....*12*
https://dx.doi.org/10.4314/evj.v23i1.2

Prevalence of Bovine Hydatidosis and its Economic Importance in Adama Municipal Abattoir, Eastern Ethiopia
Mekuria Mandefro; Birhanu Tilahun; Yehualashet Bayu; and Tesfaheywet Zeryehun.....*24*
https://dx.doi.org/10.4314/evj.v23i1.3

Comparative diagnostic techniques in ruminant Fasciolosis: fecal sedimentation, indirect ELISA, liver inspection and serum enzyme activities
Meskerem Adamu, Abebe Wossene, Getachew Tilahunand Asoke Kumar Basu.....*42*
https://dx.doi.org/10.4314/evj.v23i1.4

Prevalence, risk factors and antimicrobial susceptibility profile of ***Salmonella*** isolated from dogs of Ambo, Bako and Gojo towns of West Shoa, Ethiopia
Endrias Zewdu Gebremedhin, Sisay Miheretu, Lencho Megersa, Edilu Jorga Sarba, Getachew Kebebew and Solomon Shiferaw.....*59*
https://dx.doi.org/10.4314/evj.v23i1.5

Prevalence and Risk Factors of Ectoparasites in Small Ruminants in and around Haramaya University, Eastern Oromia Region, Ethiopia
Adugna Abera and Tsegay Tkue Gebrewahd.....*78*
https://dx.doi.org/10.4314/evj.v23i1.6

Isolation, DNase-cross-Coagulase test and antimicrobial resistance test on ***Staphylococcus*** along beef abattoir line in Addis Ababa Ethiopia
Adem Hiko.....*90*
https://dx.doi.org/10.4314/evj.v23i1.7

Ethno-veterinary medicine: A potential alternative to animal health delivery in Wolmera district, Oromia Region, Ethiopia
Yohans Abera and Belay Mulate *111*
https://dx.doi.org/10.4314/evj.v23i1.8

Guidelines for authors.....131

Carcass characteristics of draught cattle released for beef in Eastern Ethiopia

Ewonetu Kebede Senbeta and Ashenafi Getachew Megersa

School of Animal and Range Sciences, Haramaya University

+251912927403, ewonetu2011@gmail.com, P.O. Box 138, Dire Dawa, Ethiopia

Abstract

Most cattle used for beef production in Ethiopia are Zebu breeds. Usually released for beef when they are aged for plowing and in poor body condition. However, there is little available information on carcass yield and percentage earned from these cattle. Therefore, the study was conducted to evaluate the carcass yield and the percentage of cattle released for beef after used in draught power. This study used 280 heads of male indigenous draught cattle released for beef. Each cattle were randomly measured for carcass and non-carcass components. Body weight was recorded as animals arrived. Hot carcasses were weighed and cold carcasses were estimated at 0.98 of the hot carcass weight. Dressing proportions were calculated from the ratio of hot carcass weight to slaughter weight. Descriptive statistics for carcass yield, edible and offal components were analyzed by SPSS. The average slaughter weight, hot carcass weight, dressing and shrinkage percentage recorded in this study was 247.93±5.27, 90.98± 2.11, 36.98±0.94 and 0.74±0.02, respectively. The amount of total deboned lean meat was 60.38kg (24.35% of the slaughter body weight). The price of live animals and the amount of carcass and other edible parts attained from it, is not worthy of comparison and there was a loss of 402.66± 0.29 Birr per each cattle. Therefore, draught cattle released for beef after draught power should be fattened either by farmers or beef farm to recover their body weight loss due to agricultural work load.

Keywords: Carcass; Drought cattle; External offal; Internal organ

Introduction

Ethiopia produces about 0.33 million tons of meat annually from cattle (CSA, 2008). Average carcass weight of cattle is 108 kg/head (Negassa *et al.,* 2011), while Ethiopians consume about 8-13.9 kg of meat per capita annually, being

lower than the African and the world per capita averages, which are 27 and 100 kg/year, respectively (Ayele and Peacock, 2003; Betru and Kawashima, 2009; FAO, 2009). Most farmers in Ethiopia usually sell draught cattle after the plowing season when they are in poor condition and aged for drafting purposes (Teshager *et al.,* 2013; Yesihak and Edward, 2014*).* Carcass composition (proportions of muscle, fat and bone) largely determines carcass value (Pesonnen *et al.,* 2012). A high proportion of muscle with a low proportion of bone and an optimum level of fat represents a superior carcass (Oprzadek *et al*., 2001). Yesihak and Edward (2014) reported carcass weight of 155.02 ± 0.83 kg in wet season and 119.56 ± 0.89 kg of hot carcass in dry season for indigenous cattle of Ethiopia. The origin of animals, carcass characteristics and its quality are important criteria for butchers and consumers when it comes to making purchasing decisions (Carlos *et al.,* 2009). Characterizing carcass traits of cattle used for agricultural cultivation is important to develop an appropriate improvement strategy of the sector. Carcass traits broadly describe carcass quality (composition) and carcass quantity (Aynalem *et al.,* 2011). Carcass quantity traits comprise of pre-slaughter live weight, hot carcass weight and dressing percentage (Pariacote *et al.,* 1998). However, the carcass characteristics differ among breeds and are influenced by the plan of nutrition and production system (Keane and More O'Ferrall, 1992). Selection for these traits is greatly influenced by the market demand. The ability of the producers and buyers of beef cattle is to relate objective live animal to carcass characteristics which is essential for optimum production and value-based trading systems (Afolayana *et al*., 2002). This will also enable processors to determine returns from carcass processing and it may increase the rate of genetic gains in meat production traits. The traditional mixed crop-livestock farming practice in Ethiopia mainly demands male cattle to serve as draught animals (IGAD, 2010). Draught cattle are normally released for beef when they are aged for plowing. However, there is little available information with regard to carcass yield and the percentage contribution of various components of edible and offal carcass of cattle released for beef after draught powers. Therefore, this study was conducted to evaluate the carcass yield and percentage of various edible and offal components of cattle released for beef after draught powers.

Material and methods

Study area

The study was conducted at Haramaya University in Haramaya district, Eastern Ethiopia ,which is 5, 17, 40 and 527 km from Haramaya town, Harar city, Dire Dawa city administration and east of Addis Ababa respectively. The study area is found at an elevation of 2000m above sea level, located at 041°59'58" latitude and 09°24'10" longitudes. It receives an average annual rain fall of 900mm. With respect to Agro-ecological zones, 66.5% is midland and 33.5% is lowland. It has about 63,723 cattle, 13,612 sheep, 20,350 goats, 15,975 donkeys, 530 camels and 42,035 chickens (Unpublished data from Haramaya Agricultural office, 2017/2018).

Sampling method and sample size determination

Only male draught cattle after worked up in agricultural cultivation in different regions of Eastern Ethiopia were directly supplied by unions to Haramaya University for consumption at students' cafeteria. Among the received cattle, the carcass and non-carcass component of 280 individual animal was randomly measured.

Study animal management

Experimental animals were purchased from different markets in eastern Ethiopia namely Hirna, Chelenko, Haramaya, Kulubi and then transported by trekking to the University where they were slaughtered. According to the information from the supplying Unions, animals did not pass through the fattening process. As well, all the slaughtered cattle were used for draft power in mixed crop-livestock production system and after the end of plowing season they were immediately released for beef purpose. Body weight was recorded as animals arrived at the University's beef farm and then they fed in group pens on hay and crop residues as *ad libitum* and sometimes supplemented students' cafeteria leftover for two weeks during the quarantine and ante mortem inspection. Before slaughtering, the animals held off feed and water for 12 to 16 hours to assure complete bleeding and ease of evisceration.

Slaughter procedure and data collection

Animals were slaughtered at the University's abattoir according to the standard procedure. Bleeding was effected by cutting the jugular vein. The head was removed at the atlanto-occipital joint, fore and hind feet removed at the carpus-metacarpal and tarsus-metatarsal joints respectively (Safari *et al.,* 2009). Following the slaughter, the carcasses yield and quantitative characteristics of edible and offal were weighed by weighing balance. Hot carcass was weighed and cold carcass weight was estimated at 0.98 of the hot carcass weight (Pesonen *et al.,* 2012). Dressing proportion was calculated from the ratio of hot carcass weight to slaughter weight, i.e.

$$\text{Cold carcass Dressing Percent} = \text{Hot Carcass Weight} \times 0.98$$

$$\text{Hot carcass Dressing Percent} = \frac{\text{Hot Carcass Weight} \times 100}{\text{Slaughter Weight}}$$

Shrinkage percentage was calculated as the loss of weight after chilling in relation to hot carcass weight. Generally, on average, each day seventeen animals were slaughtered for evaluation.

Statistical analysis

Descriptive statistics of the carcass yield, the edible and offal component of carcass was analyzed by Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, Illinois, USA, 2011).

Result

Table 1. Slaughter and carcass weights and dressing percentage of cattle used for draught power (n=280).

Parameters	Min.	Max.	Mean + SE
Slaughter weight (Kg)	200.00	300.00	247.93±5.27
Hot carcass weight (Kg)	72.50	119.00	90.98± 2.11
Cold carcass weight (Kg)	71.05	116.62	89.16±10.94
Hot carcass dressing % of LW	28.93	48.75	36.98±0.94
Cold carcass dressing % of LW	28.35	47.78	36.24±0.92
Shrinkage (%)	0.58	0.97	0.74±0.02

=Live weight, SE-Slandered Error of mean,

Table 2. Means weight (Kg) of non-carcass components of cattle used for draught power (n=280).

Offal's	Min.	Max.	Mean +SE	%
Feet (Legs)	4	6	5.17 ±0.13	2.09
Head with horn	11	16.5	13.43±0.27	5.42
Hide with tail	18	26..5	22.01±0.38	8.88
Trachea	0.50	2.00	1.18±0.07	0.48
Genital (Scrotal)	1.00	2.50	1.44±0.08	0.58
Ligaments (inedible fibers)	3.00	11.00	5.96±0.37	2.41

Table 3. Mean weight of internal organ components of cattle used for draught power (n=280).

Internal organ (kg)	Min.	Max.	Mean ± S. E	%
Heart	0.5	1.80	1.01±0.08	0.41
Liver	1.50	4.50	2.98±0.21	1.20
Lung	1.50	6.00	2.42±0.17	0.98
Spleen	0.50	2.00	0.94±0.08	0.38
Kidney	0.50	2.00	0.99±0.08	0.40

Table 4. Cost-benefit analysis for total hot carcass yield (n=280).

Variable	Min.	Max.	Mean ± SE
Purchase price/live cattle (ETB)	6400.00	9600.00	7933.71±168.52
Hot carcass sale price/ cattle (ETB)	5800.00	9520.00	7278.57±168.81
Other organs and hide Price/ cattle (ETB)	240.00	265.00	252.50
Total carcass sale price/ Slaughter cattle (ETB)	6040.00	9785.00	7531.05±168.81
Net profit/ Slaughter cattle (ETB)	-360.00	185.00	-402.66±-0.29

Note: Purchase price per Kg live weight-32 ETB, sale price per kg carcass weight-80 ETB

Table 5: Total proportion of carcass and non-carcass components.

Variable	Mean (Kg)	% (On slaughter weight basis)
Deboned meat	60.38	24.35
Bone without meat	11.34	4.57
Bone with residual meat	30.6	12.35
All internal organs*	8.27	3.34
All non-carcass components+	8.20	3.31

***=** *Heart, Liver, Lung, Spleen,* & *Kidney.* **+=** *Legs, Head with horn & tongue, Skin with tail, Trachea, Scrotal,* & *Ligaments (inedible fiber)*

Discussion

The average slaughter weight recorded in this study (Table 1) was higher than Sanga (202kg) and the humpless West African shorthorn (WASH) (162kg) cattle slaughtered at local abattoir in Ghana (Teye and Sunkwa, 2010). However; it is less than weight of Zebu (309kg), Nguni (324kg) and Tuli (418kg) cattle slaughtered in South Africa (Strydom, 2008). Mengistu *et al*. (2013) also reported higher slaughter weight 489.7±10.6 and 274.9±10.6 for Holstein Frisian and Boran crossbred oxen and Ethiopian highland Zebu cattle respectively. The lower slaughter weight reported in this study is consistent with Teshager *et al.* (2013), and Yesihak and Edward (2014*)* who reported that most of the local cattle used for meat production in Ethiopia are usually supplied to market after the plowing season when they are in poor body condition and older in age for the draught purposes. The average hot carcass weight in this finding was less than the carcass weight reported for Boran cattle (98.2-135.2kg) in Ethiopia (Lemma *et al*., 2007), Zebu breed (155.9kg) in Ghana (Teye and Sunkwa, 2010), Ogaden cattle (163-182kg) in Ethiopia (Mekasha *et al.,* 2011), Nguni (181kg) and Tuli (241 kg) in South Africa (Strydom, 2008), Arsi cattle (163.13 ± 29.09kg) in Ethiopia (Gebeyehu *et al.,* 2018), Bali and Ongole crossbreed (PO) cattle (125.07 ± 21.47 kg) (Haryoko and Suparman, 2009). Teye and Sunkwa (2010) reported higher and lower hot carcass weight than the result obtained in this study for Sanga (95.3kg) and WASH (74.1Kg), respectively. Moreover, Mengistu *et al*. (2013) reported higher hot carcass weight (248.5±5.5) and (141.1±5.5) for Holstein Frisian and Boran crossbred oxen and Ethiopian highland Zebu cattle respectively. Yesihak and Edward (2014*)* also reported relatively higher carcass weight for different indigenous cattle breeds at Adama (161.26 + 1.05 kg), Hawassa (142.46 ± 1.10kg), Kombolcha (95.63 ± 0.46) and Mekelle (136.15 ± 1.17 kg) abattoirs in Ethiopia. Further, the amount of total deboned lean meat obtained in this study (Table 5) is lower than 68 and 68.2kg that was the study reported by Gebeyehu *et al* (2018) and Bedhane and Dadi (2016) respectively. This lower result was due to the low slaughter weight and previous agricultural work load or stress.

The hot dressing percentage for cattle released for beef after draft powers in this experiment was less than the results reported by Teye and Sunkwa (2010) for WASH (45.9%), Sanga (47.6%), and Zebu (52.1%). Mengistu *et al* (2013) also reported higher dressing percentage for Holstein Frisian and Boran crossbred oxen (51.3±0.8), for Ethiopian highland Zebu cattle (51.4±0.8), for non-working (51.1±0.9) and worked Ethiopian cattle breeds (51.6±0.6) respectively.

Moreover, many scholars reported higher hot dressing percentage for different cattle breeds such as 47.78 ± 2.82% for PO cattle (Haryoko and Suparman, 2009), 47.49% for Boran and 44.93% for Kereyu breeds (Mohammed *et al*., 2008), 53.15 ± 5.75% for Arsi cattle (Gebeyehu *et al*., 2018). This disparity in slaughter weight, carcass yield and dressing percentage could perhaps be due to differences in environment, breed type, age of slaughter, the level of fattening, agricultural work load (draught power) and management given which were entirely absent in the present study.

Shrinkage values obtained was lower than (Table 1) that obtained by Eltahir (1994), Mohamed (1999), Elkhidir (2004) and Mohammed (2004). As well, Mohammed *et al* (2015) reported higher shrinkage percentage of 2.78, 2.04, 2.78 and 1.92 for Sudan Baggara Zebu bulls finished on urea-treated contained 0, 10, 20 and 30% of treated bagasse respectively. The lower in shrinkage value might be due to poor body condition (less expected fat deposition in carcass of oxen used in draft power due to work stress) and low slaughter weight of the experimental oxen and this is confirmed to the ideas of Mohamed (1999) and Mohammed *et al* (2015) who stated that moisture evaporation was reduced with increasing fat deposition in the carcass and that will affect chilling shrinkage.

The external offal's like head and legs (Table 2) were comparable to the result reported for Zebu cattle which were 13.05 and 4.77kg, respectively. However, lower results reported by Teye and Sunkwa (2010) for Sanga and WASH cattle heads (10.2 and 8.7kg) and legs (3.7 and 3.18kg). Likewise, Mengistu *et al*. (2013) reported lower weight for Ethiopian highland Zebu hide (19.2±0.9) and head (14.1±0.5), but he reported higher weight for Holstein Frisian and Boran crossbred oxen hide (32.8±0.9), head (24.1±0.5), and legs (10.1±0.2). Moreover, Mohammed *et al*. (2015) reported lower weight in all treatments for feet, genitalia, head, and hide of Sudan Baggara Zebu bulls finished on urea-treated contained 0, 10, 20 and 30% of treated bagasse. This study's result is in consistent with Terry *et al* (1990) who reported that hide of Bos indicus cattle is generally heaviest.

The proportion of internal organ components like heart, liver, lung, spleen and kidney are indicated in Table 3. This finding is almost as good as to the study result reported for Zebu cattle heart (1.03), liver (3.85), lung (3.48), spleen (0.99) and kidney (0.597) (Teye and Sunkwa, 2010). Besides, the same authors reported lower result for the same internal organs of Sanga and WASH cattle

(0.75 & 0.65, 2.6 & 2.38, 1.66 & 1.6, 0.6 & 0.56 and 0.48 and 0.42 respectively). Moreover, Mohammed *et al.* (2015) reported lower weight in all treatments for heart, liver, kidney, and spleen of Sudan Baggara Zebu bulls finished on urea-treated contained 0, 10, 20 and 30% of treated bagasse.

Simple economic profit calculation was done from the purchase price of live draught cattle and sale from its carcass and other edible parts (Table 4). This study result discloses that, the price of live animals and the amount of carcass and other edible parts attained from it, is not worthy of comparison and there was a loss of 402.66 ± 0.29 Birr per slaughtered animal. This is due to high proportion of offal or ligaments (Table 2), poor body conditions (might be due to previous work stress), blood drained and inedible bone thrown. Therefore, it is plain that profit is acquest barely from draught power animals if the students' cafeteria accustomed their flesh for sale. And the students' cafeteria is obliged to postulate the shamble to quell a large number of cattle to intercept the students' penury of flesh for food.

Conclusion

The average slaughter and hot carcass weight, and dressing percentage recorded for draught cattle in this study was 247.93±5.27, 90.98± 2.11 and 36.98±0.94 respectively, while the shrinkage percentage value was 0.74±0.02. The amount of total deboned lean meat obtained was 60.38kg (24.35%). The price of live animals and the amount of carcass and other edible parts attained from it, is not worthy of comparison and there was a loss of 402.66± 0.29 Birr per slaughtered animal. Generally, the carcass yield earned from cattle used in this study is low and not profitable. Therefore, draught cattle released for beef should be fattened either by the farmer themselves or at beef farm in order to recover their body weight due to agricultural work load and to minimize number of animals scarified every day. As well, detailed study should be conducted to evaluate rib eye area, fat thickness and primal cuts for leg, lion, rack, shoulder, neck, breast and shank and also meat quality needs to be addressed so as to evaluate the effect of work performance on carcass quality of draught animals released for beef purpose.

Acknowledgements

The authors would like to thank Haramaya University and the University's enterprise development directorate office for financial support to conduct this

study. In addition, the authors are grateful to the University's beef farm and abattoir staffs that provided assistance during experimental work.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Afolayana, R. A., Deland, M.P., Rutley, D.A., Bottema, C.D., Ewers, A.L., Ponzoni, D. R. and Pitchford, W.S., 2002. Prediction of Carcass Meat, Fat and Bone Yield across Diverse Cattle Genotypes Using Live-Animal Measurements. *Anim. Prod. Aust.*, 24, 13-16.

Ayele, S., Assegid, W., Jabbar, M. A., Ahmed M.M. and Belachew, H., 2003. Livestock marketing in Ethiopia: A review of structure, performance and development initiatives. Socioeconomics and Policy Research Working Paper 52. International Livestock Research Institute (ILRI), Nairobi, Kenya. 35.

Aynalem, H., Workneh, A., Noah, K., Tadelle, D. and Azage, T., 2011. Breeding strategy to improve Ethiopian Boran cattle for meat and milk production. IPMS of Ethiopian Farmers Project Working Paper 26. Nairobi, Kenya, ILRI.

Bedhane, M. and Dadi, H., 2016. Growth and Slaughter Characteristics of Ethiopian Boran Breed Bull. *Int. J. Livest. Res.,* 6, 41-50.

Betru, S. and Kaawashima, H., 2009. Pattern and determinants of meat consumption in urban and rural Ethiopia. *Livest. Res. Rural Dev.*, P. 21. Article # 143.

Carlos, O., Pena, F., Garcia, A., Perea, J., Martos, J., Domenech, V. and Acero, R., 2009. Carcass characteristics, fatty acid composition, and meat quality of Criollo Argentina and Braford steers raised on forage in a semi-tropical region of Argentina. *Meat Sci.*, 81, 57-64.

CSA, 2008. Ethiopian Statistical Agency. CSA, Addis Ababa, Ethiopia.

Elkhidir, I. A., 2004. Utilization of sugar-cane bagasse for fattening cattle. Ph.D thesis. University of Khartoum.

Eltahir, I. E., 1994. Beef production potential of Baggara and 50% Friesian crossbred. MSc. Thesis. University of Khartoum.

FAO, 2009. Production year book. FAO, Rome, Italy. http://faostat.fao.org/default.aspx.

Haryoko, I. and Suparman, P., 2009. Evaluation of Carcass Production of PO Cattle Based on Heart Girth Measurement, Body Condition Score and Slaughter Weight. J. Anim. Prod., 11, 28-33.

Gebeyehu, A. W., Yousuf, M., Sebsibe, A., 2018. Evaluation of microbial load of beef of Arsi cattle in Adama Town, Oromia, Ethiopia**.** International Conference on Food safety and Hygiene, September 06-08, 2018 | Edinburgh, Scotland**.**

IGAD, 2010. The contribution of Ethiopian Livestock to the Economies of IGAD Member States. IGAD LPI Working paper No. 02-10.

Keane, M.G. and More O'Ferrall, G.J., 1992. Comparison of Friesian, Canadian Hereford × Friesian and Simmental × Friesian steers for growth and carcass composition. *Anim. Prod.*, 55, 377-387.

Lemma, T., Geleta, T., Sisay, A. and Abebe, T., 2007. Effects of four different basal diets on the carcass composition of finishing Ethiopian Boran bulls. *J. Cell Anim. Biol.*, 1, 015-018.

Mekasha, Y., Mengistu, U., Mohammed, Y.K. and Merga, B., 2011. Effect of strategic supplementation with different proportion of agro-industrial by-products and grass hay on body weight change and carcass characteristics of tropical Ogaden bulls (Bos indicus) grazing native pasture. *Afr. J. Agric. Res.*, 6, 825-833.

Mengistu, A., Zewdie, W., Yohannes, G. and Kassahun, W., 2013. Evaluation of Friesian x Boran crossbred and Ethiopian Highland Zebu oxen with a reciprocal work effect on carcass characteristics. *Int. J. Agric. Sci., 6, 1016-1020.*

Mohamed, H. K., 1999. The effect of different dietary energy levels on performance, carcass characteristics and meat quality of Baggara bulls. Ph. D. Thesis. University of Khartoum.

Mohammed, A. M., 2004. Effect of slaughter weight on meat production heifers. Ph. D. Thesis. University of Khartoum.

Mohammed, H. A., Salih A. B., Abdelrahman, M. M. and AbdElnasir, M. A. F., 2015. Carcass Yield and Characteristics of Sudan Baggara Zebu Bulls Finished on Urea-Treated Sugar-Cane Bagasse, *Inter. J. Anim. Biol.*, 1, 11-16.

Negassa, A., Rashid, S. and Gebremedhin, B., 2011. Livestock Production and marketing. Ethiopia Strategy Support Program II (ESSP II). ESSP II Working P.26.

Oprządek, J., Dymnicki, E., Oprządek, A., Słoniewski, K., Sakowski, T. and Reklewski, Z., 2001. A note on the effect of breed on beef cattle carcass traits. *Anim. Sci. Pap. Rep.,* 19, 79–89.

Pariacote, F., Van Vleck, L.D. and Hunsley, R.E., 1998. Genetic and phenotypic parameters for carcass traits of American Shorthorn beef cattle. *J. Anim. Sci.*, 76, 2584-2588.

Pesonen, M.M.H. and Arto, H., 2012. Effect of breed on production, carcass traits and meat quality of Aberdeen Angus, Limousin and Aberdeen Angus x Limousin bulls offered a grass silage-grain-based diet. *Agr. Food Sci.*, 21, 361-369.

Safari, J. G., Mushi, D. E., Mtenga, L. A., Kifaro, G. C. and Eik, L. O., 2009. Effects of concentrate supplementation on carcass and meat quality attributes of feedlot finished Small East African goats. *Livest. Sci.*, 125, 266-274.

Strdom, P. E., Frylink, L., Van der Westhuien, J. and Burrow, H. M., 2008. Growth performance, feed efficiency and carcass and meat quality of tropically adapted breed types from different farming systems in South Africa. *Aust. J. Exp. Agr.,* 48, 499-507.

Terry, C.A., Kenapp, R.H., Edwards, J.W., Mies, W.L., Savell, J.W. and Cross, H.R, 1990. Yields of by-products from different cattle types. *J. Anim. Sci.*, 68, 4200-4205.

Teshager, A., Belay, D. and Taye, T., 2013. Traditional Cattle Fattening and Live Animal Marketing System in Different Agro-Ecologies of Ilu Aba Bora Zone, Oromia, Ethiopia. *Global Veterinaria,* 10, 620-625.

Teye, G.A. and Sunkwa, W.K., 2010. Carcass Characteristics of Tropical Beef Cattle Breeds (West African Shorthorn, Sanga and Zebu) in Ghana. *Afr. J. Food Agric. Nutr. Dev.,* 10, 2866-2883.

Yesihak, Y. M. and Edward, C. W., 2014. Ethiopian beef carcass characteristics. *Afr. J. Agric. Res.,* 9, 3766-3775.

Seroprevalence of bovine leukemia virus infection in contrasting farming systems in Kenya

Kathambi E. K.[1], Gitau G. K.[1], Muchemi G. M.[1], VanLeeuwen J.[2]and Kairu-Wanyoike S.[3]*

[1]Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi, Nairobi

[2]Atlantic Veterinary College, 550 University Avenue, C1A 4P3, Canada

[3]Central Veterinary Investigation Laboratories, Private bag 00625, Kangemi, Nairobi

[*]Corresponding author email: kiuguek@gmail.com phone number: +254 715 819 476

Abstract

Enzootic bovine leukosis (EBL) is a worldwide disease of cattle caused by the bovine leukemia virus (BLV) and clinically characterised by occurrence of multiple lympho-sarcomas. In Kenya, cases of bovine lympho-sarcomas have been reported but limited information available on prevalence and distribution of BLV infection in the country. The objectives of this cross-sectional study were to estimate the seroprevalence of BLV infection in Kenya and how the seroprevalence is affected by different livestock farming systems. In 2016, 1383 bovine serum samples were randomly collected from 14 counties which were purposively selected to represent 3 livestock farming systems in the country. The sera were tested for the presence of antibodies against BLV using the IDEXX anti–BLV indirect ELISA test. An overall seroprevalence of 7.6% (95% CI: 6.3% - 9.1%) BLV infection was estimated. A multivariable mixed logistic regression model, with county as a random variable controlling for clustering, identified age and farming system as significant risk factors associated with BLV seropositivity. Zero-grazing (0.6%), ranching (4.4%) and pastoral systems (18.3%) differed in seroprevalence. Cattle under 1 year of age had a prevalence of 6.4%, while cattle over 1 year of age had a prevalence of 7.9%. BLV infection was present across the three farming systems but in only five of the fourteen counties assessed. This information contributes to designing effort on control programs of BLV infection in Kenya. Further research should be carried out to determine the frequency of clinical cases of EBL and the impact on the livestock industry in Kenya.

Keywords: Bovine Leukemia Virus; Cattle, Seroprevalence; Kenya

Introduction

Enzootic bovine leukosis (EBL) is a lympho-proliferative disease of cattle caused by the bovine leukemia virus (BLV) which is a delta retrovirus in the retroviridae family and is notifiable according to the World Organization for Animal Health (Stear, 2005). Natural BLV infection has been confirmed in cattle and water buffaloes (Hald and Baggesen, 2013). Trans-placental transmission has been reported to account for 10–25% of infections; natural transfer of BLV infected cells between animals during processes such as parturition is fairly common. Artificial transmission frequently occurs through blood contaminated instruments such as needles, surgical equipment and rectal gloves (Mekata *et a*l., 2015). Large numbers of blood-sucking insects, such as Tabanids, have been shown to transmit the virus mechanically (Kobayashi *et al*., 2014). Pro-viral DNA can be isolated in semen and milk of the infected animals (Santos *et al*., 2007).

Bovine leukemia virus infects B-lymphocytes which proliferate through mitosis. About 70% of the infected animals act as carriers and do not manifest clinical signs or a change in the circulating lymphocyte counts. At this stage, antibodies against the virus and the pro-viral DNA can be detected using serology and Polymerase Chain Reaction (PCR), respectively (Rola and Kuzmak, 2002; Hald and Baggesen, 2013). With time, about 30 to 50% of the infected animals develop persistent lymphocytosis which is a polyclonal proliferation of B-cells (Stear, 2005)**.** Elevated numbers of circulating B-lymphocytes (over 10,000/mm$^{3)}$ can be observed at this stage using hematology (Hald and Baggesen, 2013). About 5% of the infected animals develop the clinical form of bovine leukosis, with clinical signs including; enlarged lymph nodes, in appetence, weight loss and general weakness (Stear, 2005). On post-mortem, malignant lympho-sarcomas are observed in multiple organs of the body, especially lymph nodes, spleen, mesentery and uterus (Stear, 2005).

Enzootic bovine leukosis has no treatment or vaccine available at present (Stear, 2005). Approaches to control and eradicate the disease involve testing cattle serologically, and then eliminating, segregating or managing the infected cattle (Rodriguez *et al*., 2011). Zoonotic importance of bovine leukemia virus has been studied widely but no conclusive evidence of transmission has been established (Baltzell *et al*., 2009; Buehring *et al*., 2014).

The economic losses associated with BLV positivity include; increased heifer replacement, condemned carcasses, decreased reproductive efficiency, decreased milk production, cattle deaths and inability to export cattle and their products to regions with strict enzootic bovine leukosis control measures, such as the European Union (Ott *et al*., 2003; Rhodes *et al*., 2003).

Presently, BLV infection affects cattle herds globally, with herd-level prevalence being as high as 88% and 91% in dairy herds in the United States and Canada, respectively, and about 30% of animals infected in those herds (Ott *et al*., 2003; Nekouei *et al*., 2015). In Uganda, a 17% animal-level prevalence has been reported (Azuba *et al*., 1994), while other African countries, such as South Africa, Namibia, Nigeria and Tanzania, reported animal-level prevalences of 12.6%, 12.3%, 4.2% and 36%, respectively (Adu and Olson, 1981; Kaura and Hbschle, 1994; Schoepf *et al*., 1997; Ndou *et al*., 2011).

In Kenya, 35 cases of bovine lympho-sarcoma were reported on post-mortem in the department of Pathology, Microbiology and Parasitology at the Faculty of Veterinary Medicine in the University of Nairobi over the last 28 years (Wandera *et al*., 2000). However, prevalence, distribution and risk factors of BLV infection in Kenya have not been described. This study was aimed at estimating the seroprevalence and identify potential risk factors associated with the presence of BLV infection in different livestock farming systems in Kenya.

Materials and methods

Study area

Bovine sera used in this study were collected from cattle in 14 counties in Kenya namely: Laikipia, Garissa, Marsabit, Narok, Nyeri, Nakuru, Nyamira, Kiambu, Kakamega, Homabay, Nandi, Kwale, Murang'a and Machakos.

Study design

The Bovine serum samples were collected as part of a larger cross-sectional surveillance study for trade sensitive diseases that was carried out between July and October 2016 jointly by the Department of Veterinary Services in the Ministry of Agriculture, Livestock and Fisheries in Kenya, and the Inter-Governmental Authority on Development (IGAD) across the 47 counties of Kenya.

Sampling technique and sample size determination

Briefly, stratified multi-stage sampling method was adopted whereby administrative locations (lower than divisions and headed by a chief) in each of the counties were purposively selected. From these locations, households were randomly selected, and animals randomly sampled from each of the households. The number of households selected per location was dependent on the number of households in the location and the number of animals per household. Each location was considered a site, and a total of 14 bovine samples were collected from each site.

For this study, a minimum sample size of 384 per farming system (a total of 1152 samples) was determined using the formula described by (Dohoo *et al.*, 2009) using a hypothetical BLV prevalence of 50%, 95% confidence, and 5% precision. However, there were enough kits to test 1383 samples. A total of 1383 cattle were randomly selected from 14 counties that were purposively selected to represent zero-grazing, ranching and pastoral livestock farming systems in the country. Cattle kept in other livestock farming systems were excluded from the study.

Data collection

In addition to the cattle identification, breed, age, sex, farming system and county of origin of each cattle were recorded. Farming systems were categorized into: 1) zero-grazing, where cattle do not graze, and are often segregated within a farm to ensure different and appropriate management to different animal cohorts (young stock, milking cattle, dry cattle, bulls); 2) ranching, where cattle graze together but do not mingle with cattle from other farms; and 3) pastoral, where cattle migrate long distances to graze on individual and communal lands, co-mingling with cattle from other farms.

Laboratory analysis

The serum samples were tested for the presence of antibodies against BLV using the IDEXX anti–BLV indirect ELISA test (IDEXX Laboratories, Inc., Westbrook, Maine, USA), in the serology laboratory at the Central Veterinary Investigation Laboratories in Kabete, Nairobi. The test was conducted according to the manufacturer's instructions. The optical density was measured using the Halo LED 96 DYNAMIC ELISA Reader at 450nm. The ELISA kit used

in this study had a sensitivity of 100% and a specificity of 97% (Tirziu *et al*., 2014) indicating good reliability of the testing method.

Data handling and analysis

The results were entered into Microsoft Excel® 2013 (Microsoft, Sacramento, California, USA) and analysed using Stata 14 (StataCorp, College Station, Texas, USA). Seroprevalence of BLV infection (expressed as a percent positivity) was described in the different categories of age, sex, breed, farming system and the county of origin. Logistic regression was used to determine the possible risk factors for the presence of BLV infection in cattle in Kenya, initially in univariable analyses for each possible risk factor. Mixed logistic regression was used to determine the possible risk factors of BLV seropositivity in a multivariable model, controlling for confounding and with county of origin analysed as a random effect to account for clustering of the data at this level. Pearson's correlation coefficient was used to determine correlations between risk factors in order to guide the model-building process by eliminating collinear predictors ($r \geq 0.5$). Total variation and intra-class correlation (ICC), which provided the proportion of variance at the county level, were calculated using the latent variable approach (Dohoo *et al*., 2009). Only main effects were retained in the final model at a significance level of $P \leq 0.05$, and interactions between main effects were investigated in the final model. Testing for confounding by variables not in the final model was explored by comparing changes in model coefficient estimates with and without the confounder and a change of ≥30% on the coefficient was used to indicate a confounder. Goodness-of-fit of the model was assessed and a likelihood ratio test was used to determine the superiority of a mixed logistic model over a fixed effect logistic model.

Results

The overall seroprevalence was 7.6% with 105 of the 1383 sera samples testing positive for BLV antibodies (95% CI: 6.3 - 9.1). The prevalence was only slightly higher in older cattle versus yearlings and in male cattle versus females ($p > 0.05$). Indigenous cattle appeared to have a higher prevalence than exotic breed cattle on univariable analyses. The prevalence of BLV infection in the farming systems ranged from 18.3% in the pastoral system to 0.6% in the zero-grazing system. Five counties in the study (Nakuru, Nandi, Laikipia, Marsabit and Garissa) had a prevalence that ranged from 2.9% (Nakuru) to

37.5% (Garissa), while the other counties had no positive animals for BLV infection, with an average, minimum and maximum of 70, 14 and 210 animals tested per county in those counties with no positives, respectively.

Table 1: Seroprevalence of BLV infection in cattle kept in three contrasting farming systems in Kenya in 2016.

Factor	Category	No. Positive/ No. Tested	Prevalence (%)	Odds Ratio	95% Confidence Interval	p-value
Age (years)	≤1 year	19/291	6.5	Reference	Reference	Reference
	>1 year	86/1092	7.9	1.12	0.84, 1.51	0.442
Sex	Female	78/1069	7.3	Reference	Reference	Reference
	Male	27/314	8.6	1.11	0.85, 1.44	0.444
Breed	Exotic	4/444	0.9	Reference	Reference	Reference
	Indigenous	101/939	10.8	4.40	2.47, 7.82	<0.0005
Farming system						<0.0005*
	Zero-grazing	4/638	0.6	Reference	Reference	Reference
	Pastoral	90/492	18.3	7.73	4.34, 13.93	<0.0005
	Ranching	11/253	4.3	3.10	1.60, 6.01	0.001
County						<0.0005*
	Laikipia	12/350	3.4	Reference	Reference	Reference
	Garissa	57/152	37.5	5.05	3.46, 7.39	<0.0005
	Marsabit	32/126	25.4	3.65	2.44, 5.46	<0.0005
	Nakuru	2/70	2.9	0.90	0.38, 2.14	0.808
	Nandi	2/56	3.6	1.02	0.43, 2.45	0.957
	Other 9 counties	0/629	0.0		n/a	n/a
Total		1383	7.6		6.3, 9.1	n/a

**Global p-value for categorical variables*

Breed was highly and significantly correlated with farming system (r=0.622) and county of origin (r=0.563). The likelihood ratio test indicated that the mixed logistic model was better than the multivariable logistic regression model ($p \leq 0.0005$). After accounting for clustering at the county level, and adjusting for confounding by other variables in the final multivariable model, the presence of BLV infection in Kenya was significantly associated with the cattle age and the farming system that the cattle were kept in (Table 2). The odds of testing positive for BLV infection was about 2 times higher in older cattle (>1 year) than young ones. The odds of BLV infection occurring in ranched cattle were 10 times higher than in zero-grazed cattle, while the risk of BLV

infection in pastoral cattle was over 5 times higher than in zero-grazed cattle. The probability of zero-grazed adult cattle testing positive for BLV infection ranged from 0.01% to 2.42% (95% CI for the baseline risk among zero-grazed cattle). Given that cattle in a specific county tested positive for BLV infection, the probability of a randomly selected cattle from the same county testing positive for BLV was 64.2% (ICC=0.642). The interaction variable between the two main effects of the model was not significant and there was no additional confounding by other variables not in the final model.

Table 2: Multivariable mixed logistic regression model of risk factors associated with seropositivity of BLV infection in cattle in Kenya in 2016.

Factor	Category	Odd ratio	95% CI	p-value
Age (years)	≤1	Reference	Reference	Reference
	>1	1.79	(1.26,2.56)	0.001
Farming system				0.063*
	Zero-grazing	Reference	Reference	Reference
	Pastoral	5.44	(0.98,30.27)	0.05
	Ranching	10.38	(1.48,72.97)	0.02

**Global P-value*

Discussion

This is the first study on seroprevalence of bovine leukemia virus infection in livestock farming systems in Kenya. The overall prevalence of 7.6% was low compared to other African countries such as South Africa, Namibia, Uganda and Tanzania that reported prevalences of 12.6%, 12.3%, 17% and 36%, respectively (Adu and Olson, 1981; Azuba *et al.*, 1994; Kaura and Hbschle, 1994; Schoepf *et al*., 1997; Ndou *et al*., 2011). In South Africa, EBL outbreaks had been reported before this study was carried out, which could explain the high prevalence (12.6%) seen there (Ndou *et al*., 2011) in comparison to the 7.6% prevalence reported in the present study. Schoepf et al (1997) stipulated that the high prevalence of BLV infection in Tanzania (36%) was due to the high sensitivity of the ELISA test (CHEKIT Leukotest) used in the study; however our test sensitivity was reported at 100% (Tirziu *et al*., 2014) and the prevalence was lower thus the reason could not be used the lower prevalence in the present study. Lower infection rates have been reported in Sudan (1.5%) and Nigeria (4.2%) where the less sensitive AGID test was used (Adu and Olson, 1981; Osheik *et al*., 1988; Schoepf *et al*., 1997).

The higher risk of adult cattle (OR=1.79) testing positive for BLV infection could be attributed to the persistent nature of the infection once animals are infected and due to cumulative exposures of cattle over a lifetime (Watanabe *et al*., 2015). Similar results were observed in a study done in cattle in Iran where older cattle had a higher risk of BLV infection (Mohammadi *et al.*, 2011). A study on Yaks (*Bos mutus*) in China also indicated that the prevalence of BLV infection increased with age (Ma *et al*., 2016).

The resulting higher prevalence of BLV infection in indigenous breeds (10.8%) than in exotic breeds (0.9%) seen in our univariable results was similar to reports made from other studies in Venezuela and Argentina (Trono *et al*., 2001; Nava *et al*., 2011) . In Kenya, cattle kept in pastoral systems are mostly indigenous breeds, while zero-grazed cattle are mostly exotic breeds, which could explain the correlation between breed and farming system (r=0.622), and the removal of breed in the final model when farming system was in the model. The high prevalence (18.3%) and odds of infection (OR=5) observed in cattle kept in pastoral farming systems could be due to unrestricted contact between infected and uninfected cattle among different owners during communal grazing, which eases the transmission of BLV among these cattle, where the infection exists. Consequently, in Garissa and Marsabit Counties where pastoral farming is frequently practiced, the highest BLV prevalences of 37.5% and 25.4% were observed, respectively. Movement of pastoralists with their animals in search of pasture and water could facilitate spread of the BLV. In ranches, cattle have unlimited contact with cattle in the same ranch but not cattle from other ranches, which could explain the lower prevalence (4.4%) in comparison to pastoral system. The unlimited contact between animals within a ranch could explain the higher prevalence of BLV infection when compared to zero-grazing farms which only had a prevalence of 0.6%. In the zero-grazing farming system, the risk of BLV transmission between cattle in the same herd appears to be very low, likely due to the segregation of cattle cohorts within farms (e.g. dry cattle and young stock are usually kept away from milking cattle), which likely lowers the rate of contact between the cattle in the same farm. On zero-grazing farms, there is usually no exposure to cattle from other farms. Zero-grazing livestock systems usually have higher inputs and better management than ranching and pastoral systems, and thus, cattle in this system may have better resistance to diseases.

Laikipia county, with a high number of ranches, had a moderately high prevalence of 3.4%. Of the 10 tested counties that practiced zero-grazing livestock farming, only Nandi (3.6%) and Nakuru (2.9%) had cases of BLV infection.

A limitation of this study is that we only had limited data at the animal level and at the county/system level, since we obtained serum samples from another study. We did not have herd level data at all on the cattle tested; therefore, we cannot say anything about herd-level prevalence or herd-level risk factors. We also could not adjust the prevalence for sampling weights without this herd-level information. With the limited data, we could only control for confounding for those variables with data. Future studies should collect and interpret herd level data and more animal level data to allow testing of hypotheses on the important mechanisms of BLV transmission.

Conclusion

Bovine leukosis infection was found to be present across the three farming systems assessed and in five of the fourteen selected counties in Kenya. Cattle kept in pastoral farming systems had the highest prevalence, while zero-grazed cattle had the lowest prevalence. Similarly, in counties where pastoral farming is largely practiced, a larger number of cattle tested positive for BLV relative to the other counties. The Kenyan local breeds had a higher prevalence than the exotic breeds; however, this finding is likely a function of zero-grazing farmers preferring exotic breeds over local breeds. Further research should be carried out to determine: a) BLV seroprevalence in other Kenyan counties; b) the frequency of clinical cases of enzootic bovine leukosis; and c) the economic impact of BLV in the livestock industry in Kenya, along with important management risk factors to determine recommendations for reduced transmission relevant to Kenyan management systems.

Acknowledgments

This work was supported by the University of Nairobi; Department of Veterinary Services, and Ministry of Agriculture, Livestock and Fisheries in Kenya; the Inter-Governmental Authority on Development (IGAD); the Central Veterinary Investigation Laboratory in Kabete, Kenya; and IDEXX Laboratories Inc. (Westbrook, Maine, United States).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Compliance with ethical standards

All applicable international, national and institutional guidelines for the care and use of animals were followed.

Conflict of interest statement

The authors declare they have no financial or personal relationship(s) that may have inappropriately influenced them to write this article.

References

Adu, F.D., and Olson, C., 1981. Bovine leukosis in Nigeria: a preliminary study. *Bull. Anim. Heallth. Prod. Afr*., 29, 255-258

Azuba, R.B., Zieger, U. and Schmidt, F.W., 1994. A prevalence study of bovine leukaemia virus infection in slaughtered cattle in selected areas in Uganda. *Bull. Anim. Health. Prod. Afr.,* 42, 13-17

Baltzell, K., Shen, H.M. and Buehring, G.C., 2009. Oncogenic viruses in nipple aspirate fluid: biomarkers for breast cancer risk assessment? BMC. Proc., 2009; 3(Suppl 5): S4. doi: 10.1186/1753-6561-3-S5-S4

Buehring, G.C., Shen, H.M., Jensen, H.M., Choi, K.Y., Sun, D.J. and Nuovo, G., 2014. Bovine leukemia virus DNA in human breast tissue. *Emerg. Infect. Dis.,*. 20, 772-782, doi: 10.3201/eid2005.131298

Dohoo, I.R., Martin, S.W. and Stryhn, H., 2009. Veterinary Epidemiologic Research, (Charlotte, P.E.I., Canada: VER, Inc., c2009; 2nd ed)

Hald, T. and Baggesen, D.L., 2013. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the Public Health Hazards to be Covered by Inspection of Meat (Bovine Animals), (European Food Safety Authority, Denmark, Europe)

Kaura, H.T. and Hbschle, J.B., 1994. The occurrence of enzootic bovine leukosis (EBL) in Namibia - an epidemiological study. *Dtsch. Tierarztl Wochenschr.* 101, 66-67

Kobayashi, S., Hidano, A., Tsutsui, T., Yamamoto, T., Hayama, Y., Nishida, T., Muroga, N., Konishi, M., Kameyama, K. and Murakami, K., 2014. Analysis of risk factors associated with bovine leukemia virus seropositivity within dairy and beef breed-

ing farms in Japan: a nationwide survey. *Res. Vet. Sci.,* 96, 47-53. doi: 10.1016/j.rvsc.2013.11.014.

Ma, J., Zheng, W., Zhou, D., Qin, S., Yin, M., Zhu, X. and Hu, G., 2016. First Report of Bovine Leukemia Virus Infection in Yaks (Bos mutus) in China. *Bio.Med. Res. Int*., Volume 2016, Article ID 9170167, 4 pages. doi: 10.1155/2016/9170167.

Mekata, H., Sekiguchi, S., Konnai, S., Kirino, Y., Honkawa, K., Nonaka, N., Horii, Y. and Norimine, J., 2015. Evaluation of the natural perinatal transmission of bovine leukaemia virus. *Vet. Rec.,* 176 (10), 254. doi: 10.1136/vr.102464.

Mohammadi, V., Atyabi, N., Brujeni, G.N., Lotfollahzadeh, S. and Mostafavi, E., 2011. Seroprevalence of bovine leukemia virus in some dairy farms in Iran. *Glob. Vet.,* 7(3), 305-309. ISSN 1992-6197.

Nava, Z., Obando, C., Molina, M., Bracamonte, M. and Tkachuk, O., 2011. Seroprevalence of enzootic bovine leukosis and its association with clinical signs and risk factors in dairy herds from Barinas State, Venezuela. *Rev. Fac. Cs. Vets.*, - UCV. 52 (1), 13-23.

Ndou, R.V., Sejesho, F., Dzoma, B.M., Motsei, L.E., Nyirenda, M. and Bakunzi, F.R., 2011. A serosurvey of the prevalence of enzootic bovine leukosis in the Mafikeng area of the North West Province of South Africa. *J. Hum. Ecol.,* 36, 53-55. DOI: 10.1080/09709274.2011.11906417.

Nekouei, O., Stryhn, H., VanLeeuwen, J., Kelton, D., Hanna, P. and Keefe, G., 2015. Predicting within-herd prevalence of infection with bovine leukemia virus using bulk-tank milk antibody levels. *Prev. Vet. Med.,* 122 (1-2), 53-60. doi: 10.1016/j.prevetmed.2015.10.009.

Osheik, A.A., Abbas, B., Nour Eldin, H. and Gameel, S., 1988. Bovine leukosis: serological evidence in indigenous Sudanese cattle. *Bull. Anim. Health Prod. Afr.,* 36, 310-311.

Ott, S.L., Johnson, R. and Wells, S.J., 2003. Association between bovine-leukosis virus seroprevalence and herd-level productivity on US dairy farms. *Prev. Vet. Med,.* 61(4), 249-262. doi.org/10.1016/j.prevetmed.2003.08.003.

Rhodes, J.K., Pelzer, K.D. and Johnson, Y.J., 2003. Economic implications of bovine leukemia virus infection in mid-Atlantic dairy herds. *J. Am. Vet. Med. Assoc,.* 223(3), 346-352. DOI: 10.2460/javma.2003.223.346.

Rodriguez, S.M., Florins, A., Gillet, N., de Brogniez, A., Sanchez-Alcaraz, M.T., Boxus, M., Boulanger, F., Gutierrez, G., Trono, K., Alvarez, I., Vagnoni, L. and Willems, L., 2011. Preventive and Therapeutic Strategies for Bovine Leukemia Virus: Lessons for HTLV. *Viruses.* 3(7), 1210-1248. doi: 10.3390/v3071210.

Rola, M. and Kuzmak, J., 2002. The detection of bovine leukaemia virus proviral DNA by PCR-ELISA. *J .Virol. Methods.* 99, 33-40. doi.org/10.1016/S0166-0934(01)00384-6.

Santos, M.J.D., Trono, K., Lager, I. and Wigdorovitz, A., 2007. Development of a PCR to diagnose BLV genome in frozen semen samples. *Vet. Microbiol.,* 119(1), 10-18. doi.org/10.1016/j.vetmic.2006.08.030.

Schoepf, K.C., Kapaga, A.M., Msami, H.M. and Hyera, J.M.K., 1997. Serological evidence of the occurrence of enzootic bovine leukosis (EBL) virus infection in cattle in Tanzania. *Trop. Anim. Health Prod.,* 29(1), 15-19.

Stear, M.J., 2005. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees) 5th Edn. Volumes 1 & 2. World Organization for Animal Health 2004. ISBN 92 9044 622 6. *Parasitology* 130(6), Pp. 727. DOI: https://doi.org/10.1017/S0031182005007699.

Tirziu, E., Cumpanasoiu, C., Nichita, I., Reman, G.D., Sonea, C. and Seres, M., 2014. Performance assessment of three tests applied in enzootic bovine leukosis diagnosis. *Rom Biotech. Lett.,* 19(5), 9666-9677.

Trono, K.G., Perez-Filgueira, D.M., Duffy, S., Borca, M.V. and Carrillo, C., 2001. Seroprevalence of bovine leukemia virus in dairy cattle in Argentina: comparison of sensitivity and specificity of different detection methods. *Vet. Microbiol.,* 83(3), 235-248. doi.org/10.1016/S0378-1135(01)00420-5.

Wandera, J.G., Kamau, J.A., Ngatia, T.A., Wamukoya, J.P.O. and Mulei, C.M., 2000. Bovine lymphosarcoma in Kenya. *The Kenya Veterinarian.* 20, 67-69

Watanabe, T., Inoue, E., Mori, H., Osawa, Y. and Okazaki, K., 2015. Delayed-onset enzootic bovine leukosis possibly caused by superinfection with bovine leukemia virus mutated in the pol gene. *Arch Virol.,* 160(8). doi: 10.1007/s00705-015-2457-4.

Prevalence of Bovine Hydatidosis and its Economic Importance in Adama Municipal Abattoir, Eastern Ethiopia

Mekuria Mandefro[1]; Birhanu Tilahun[1]; Yehualashet Bayu[1]; and Tesfaheywet Zeryehun[1]*

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

[*]Corresponding author: Email- tesfahiwotzerihun@yahoo.com

Abstract

Hydatidosis is a major parasitic disease condition responsible for low productivity of livestock industry in Ethiopia due to imposing poor weight gains and condemnation of organs. This study was conducted from November 2016 to April 2017 in Adama municipal abattoir to estimate the prevalence, organ distribution of the cyst, and economic importance of bovine hydatidosis. Routine meat inspection, hydatid cyst count and characterization were conducted. Out of 400 cattle slaughtered at Adama municipal abattoir, 210 (52.5%) animals were found harboring hydatid cysts. The infection prevalence was higher in lung 105(26.2%) than the rest of the organs examined. In the present study, explanatory variables (sex, age, breed, body condition and origin) were found to have statistically significant ($p<0.05$) association with the prevalence of hydatidosis. Of the total of 970 hydatid cysts counted, 529/731(72.4%) small, 89/100(89%) medium, 42/45(93.3%) large and 20/94(21.3%) calcified cysts were detected in lung. Liver harbored 194/731(26.5%) small, 11/100(11%) medium, 3/45(6.7%) large and 74/94(78.7%) calcified cysts. Out of 208 cysts subjected for fertility and viability testing 120(57.7%), 74(35.6%), 40(54%) 34(46%) and 14(6.7%) were sterile, fertile, viable, non-viable and calcified cysts, respectively. The financial loss due to organ condemnation annually and during the study period was estimated to be 894,505.612 ETB and 17,844.90 ETB, respectively. The prevalence of hydatidosis in such a high proportion implies that the disease is economically important in the study area. Therefore, there should be strict control of hydatidosis by focusing on backyard slaughter practice, unsafe offal feeding of dogs, and proper west disposal. Moreover, awareness creation on its zoonotic importance, route of transmission and the growing stray dog population need to be given special attention.

Keywords: Abattoir; Adama; Cattle; *E. granulosus;* hydatidosis

Introduction

Cystic Echinococcosis (CE) is one of the most important and commonly found parasitic zoonoses in both humans and different animals (Sadjjadi, 2006). It is a chronic zoonotic disease condition associated to infection with the larval stage (hydatidcysts) of the dog's *Echinococcus* (Elmahdi, 2004). *Echinococcus* belong to Kingdom Animalia; Phylum Platyhelminthes; Class Cestoda; Order Cyclophyllidea; Genus *Echinococcus*; Species *E. granulosus.* Two hosts (definitive and intermediate hosts) are involved in the completion of the life cycle of *E. granulosus* (Hossein *et al*., 2014).

Echinococcosis causes considerable economic losses and public health problems in many countries of the world including Europe, Asia, Africa, South America, Canada and Australia (Budke *et al*., 2006). Both cystic and alveolar *Echinococcosis* has been reported from many areas. However, cystic *Echinococcosis* is more prevalent and has been reported from all countries in the Middle East and Arabic North Africa (Rostami *et al*., 2010).

In Africa *E. granulosus* has been recognized from most countries including Ethiopia. Previous report has described the endemic occurrence of *E. granulosus* in dogs and livestock (Eckert *et al*., 2002). In North Africa an average of 600-700 cases of hydatid cyst was recorded in Algeria annually, and it is responsible for about 2000 cases of surgery each year in Tunisia. In the same country annual surgical incidence rate was 15/10000 inhabitants (Khadidja, 2011). The public health importance of Echinococcosis includes cost of hospitalization, medical and surgical fees, losses of income and productivity due to temporal incapacity to work, social consequences, due to disability and mortality (Azlaf and Dakkak, 2006).

In Ethiopia, it is the major cause of organ condemnation in most abattoirs and leads to huge economic losses from treatment costs and livestock associated production losses. Considering the economic significance caused by hydatidosis in Ethiopia, significant degrees of monetary losses were estimated at various levels in different locations. Such reported estimates indicate annual losses of 25,608 ETB (2,807.89 US$) in Tigray (Kebede *et al*., 2009c); 1,791,625.89 ETB (131,737.19 US$) in cattle slaughtered at the Hawassa municipal abattoir (Regassa *et al*., 2010); 473,173.75 ETB (51,883 US$) in cattle slaughtered at the

Debre Markos abattoir (Kebede *et al*., 2009d); and 52,828 ETB (5,869.8 US$) in cattle slaughtered at the Adama abattoir (Getaw *et al*., 2010).

Cystic Echinococcosis is also a disease of public health importance in the country (Getachew *et al*., 2012). Previous studies reveal that the prevalence of bovine hydatidiosis in cattle slaughtered in Adama municipal abattoir was 6.6% (Yemane, 1990), 46.8% (Getaw *et al*., 2010). It would be essential to have information on the current status of hydatidosis to forward pertinent recommendations. Hence, the aim of this study was to estimate the prevalence, organ distribution of the cyst, and economic importance of bovine hydatidosis in the study area.

Materials and Methods

Study area

The study was conducted in Adama municipal abattoir from November 2016 to April 2017. Adama is situated 84 km east of Addis Ababa (the capital city of Ethiopia) in Oromia Regional State at a latitude of 8° 35' 00"- 8° 36' 00" north and a longitude 39° 11'57"- 39° 21' 15" east on the escarpment of the Great Rift Valley. The town has an area of 169.9 km square and 17 kebeles (CSA, 2010; NMSA, 2016). The Adama municipal abattoir provides fresh meat for different organizations such as hotels, restaurants, University and butcheries.

Study population

The study population included all cattle brought for slaughter to Adama municipal abattoir during the study period. This population comprised of cattle of different breed, sex, age composition and originating from different districts of Adama Region, Arsi, Kereyo, Bale, Borena, Haraghe, Wolenchite, Wonji and Minjar. Number of female and exotic breed of cattle in the study population was small. During the study period (November- March) about 7700 cattle were slaughtered. The average annual slaughtered animals in the study abattoir were estimated to be 20020.

Sample size

The sample size was determined by using the formula given by Thrusfield (2005) with expected prevalence 46.8% (Getaw *et al*., 2010), level of significance 95% and absolute precision 5%.

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

Accordingly, based on the above formula a sample size of 382 was calculated, but the total number of samples analyzed in this study were 400.

Study design and methods

A cross-sectional study was undertaken from November 2016 to April, 2017. The survey was conducted based on randomized systematic sampling method on the bases of entrance to lairage and code numbers written on their body (cattle having similar code were identified using coat color and appearance of horn) for local breed of male cattle, and the first animal was selected by simple randomized sampling method. But since female and exotic cattle presented for slaughtering in the study area were few. They were all included in the study population regardless of systematic randomized method. Regular visit (three days per week) was made to conduct ante and post mortem examination of slaughtered cattle. In this, abattoir, an average of 120 and 20 cattle were slaughtered during every non-fasting and fasting day respectively (70 cattle per day) except Tuesday and Thursday since the next days are fasting days such as Wednesday and Friday respectively. The average number of animals sampled per day was 6 animals.

Ante mortem inspection

During ante-mortem examination, sex, breed, body condition and origin of each animal were recorded. The body condition of study population was classified into three main categories as poor, medium and good based on observation of muscle mass and fat cover on ribs, hip, between hooks, pins, spine and transverse processes (Nicholson and Buttrworth, 1986). The animal's age was categorized into adult (below or equal to 5 years) and old (above 5 years) based on dental eruption (Adane and Guadu, 2014).

Post mortem examination

Postmortem examination was conducted through visual inspection, palpation and systemic incision of each visceral organ particularly the lung, liver, spleen, kidney, and heart (FAO, 2003). Total numbers of hydatid cysts were counted per infected organ and the cysts were measured using a ruler and classified as small (< 5cm), medium (5-10cm) and large (> 10cm) cyst (Oostburg *et al.,* 2000; Kebede *et al.,* 2009). The cyst that showed sign of degeneration and calcification were not measured and categorized as calcified cyst.

Cyst fertility and viability testing

For confirmation of cyst fertility and viability, collected cysts were selected by purposive sampling and taken to Adama woreda parasitology laboratory. During selection, individual cysts were grossly examined for any evidence of degeneration and calcification and then the non- calcified cyst that was thought to have adequate hydatid fluid were selected. Contents of cyst was checked by transferring in to a sterile container and examined microscopically (40x) for the presence of protoscolices. Based on the presences or absence of protoscolices in hydatid fluid, cyst was identified and classified as fertile (if it has protoscolices) and non-fertile (if it has no protoscolices). The fertile cysts were further subjected to viability test where a sediment containing protoscolices was placed on the microscope glass slide and cover with cover slip and observed for amoeboid like peristaltic movement with (40x) objective. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscolices in hydatid fluid on microscope slide with the principle that viable protoscolices should completely or partially exclude the dye while the dead ones take it up (Dalimi *et al*., 2002).

Assessment of monetary loss

To study the monetary loss, due to hydatidosis, only direct loss was considered and the calculation was based on condemned organs (liver, lungs, heart, kidney and spleen). In calculating cost of condemned edible organs, 15 different meat sellers; 2 meat inspector and 10 meat consumers were interviewed randomly to establish the unit price per organ and the average organ price was determined and this price index was used to calculate the loss (Denbarga *et al*., 2011). The percent involvement of each organ was considered in the calculation. The economic significance of the parasite was determined by the following formula

set by Ogunrinade and Ogunrinade (1980). The present study didn't include indirect financial loss and loss due to the treatment of human hydatidosis.

LOC= NAS[(Plu×Cplu)+(phr×Cphr) + (pli×Cpli) + (psp×Cpsp) + (pki×Cpki)]

Where: LOC = Loss due to organ condemnation, NAS = Mean number of cattle slaughter annually, Plu = Percent involvement of lung cases. Cplu = Current mean retail price of lung, Phr = Percent involvement of heart, Cphr = Current mean retail price of heart, Pli = Percent involvement of liver, Cpli = Current mean retail price of liver, Psp = Percent involvement of spleen, Cpsp = Current mean retail price of spleen, Pkid = Percent involvement of kidney, Cpkid = Current mean retail price of kidney.

Data analysis

Data were collected and recorded in Microsoft excel 2010 spread sheet and coded accordingly. The prevalence of hydatidosis was calculated by dividing the number of hydatid positive animals with the total number of animals examined. The associations of explanatory variables (age, sex, breed, body condition scores, and origin of animals) with the disease were assessed using logistic regression. All statistical analyses were done using statistical software for social science (SPSS) version 20. Probability (P) value less than 0.05 was set as statistically significant.

Results

Active abattoir survey:

Of the 400 cattle examined during routine meat inspection at Adama Municipal Abattoir, 210(52.5%) were found to harbor hydatid cyst. The study showed that all the explanatory variables were significantly associated ($p < 0.05$) except origin of animals ($p > 0.05$), with the prevalence of hydatidosis (Table 1). The frequency of cyst varied among different origin of animals, which is highest in Kereyo followed by Wonji, Bale, Arsi and soon, respectively, even though the result was not statistically significant (Table 2).

Table 1: The occurrence of bovine hydatidosis in Adama municipal abattoir with respect to different variables

Variables	Categories	Animal examined	Negative	Positive	Prevalence (%)	p-value	OR	95% CI
Sex	Male	335	145	190	56.7%	0.00	2.9	1.7 – 5.2
	Female	65	45	20	30.8%			
Age	Adult	149	105	44	29.5%	0.00	4.7	3.0 - 7.2
	Old	251	85	166	66.1%			
Breed	Local	319	129	190	59.6%	0.00	4.5	2.6 – 7.8
	Exotic/ Cross	81	61	20	24.7%			
BCS	Poor	85	28	57	67.1%	0.00	2.3	1.5 – 3.4
	Medium	150	64	86	57.3%			
	Good	165	98	67	40.6%			
Origin	Adama	151	90	61	40.4%	0.43	0.9	0.9 - 1.1
	Arsi	63	21	42	66.7%			
	Kereyo	43	11	32	74.4%			
	Wonji	42	12	30	71.4%			
	Bale	33	10	23	69.7%			
	Harar	22	13	9	40.9%			
	Minjar	16	10	6	37.5%			
	Borena	16	12	4	25%			
	Wolnchit	14	11	3	21.4%			
Total	Total	400	190	210	52.5%			

The present study showed that the infection was higher in lung only (26.2%), followed by lung and liver together (15.2%) and liver only (9.2%), while the rest organs harbored lower frequency of cyst (table 2).

Table 2: Proportion of the infection per organs of the study animals in Adama municipal abattoir

Organ affected	Frequency	Valid percent	Cumulative percent
Lung only	105	26.2	73.8
Liver only	37	9.2	83
Lung and liver	61	15.2	98.2
Liver and heart	1	0.2	98.5
Lung and kidney	2	0.5	99.0
Lung, liver and kidney	1	0.2	99.2
Lung, liver and spleen	3	0.8	100.0
Total	400	100.0	

Cyst characterization

The load of the infection per animal varies from 1 up to 25. A total of 970 cysts were counted and 70.1%, 29.1%, 0.1%, 0.4% and 0.3% of the total cysts were found on lung, liver, heart, kidney and spleen respectively (Table 3).

In this study, 75.36% small, 10.31% medium, 4.64% large and 9.69% calcified cyst were detected. The result revealed that small cysts represent the highest proportion, while large cysts are the least in terms of their prevalence (Table 3).

Table 3: Distribution of cysts in different organs based on their size in cattle

Organ affected	Small cyst		Medium cyst		Large cyst		Calcified cyst	
	N	%	N	%	N	%	N	%
Lung	529	72.4	89	89	42	93.3	20	21.3
Liver	194	26.5	11	11	3	6.7	74	78.7
Heart	1	0.1	0	0	0	0	0	0
Kidney	4	0.6	0	0	0	0	0	0
Spleen	3	0.4	0	0	0	0	0	0
Total	731	100	100	100	45	100	94	100

Fertility and viability study

From the total 208 hydatid cyst collected at the abattoir during postmortem examination and subjected to sterility testing, 74(35.6%), 120(57.7%) and 14(6.7%) were found to be fertile, sterile and calcified respectively. Viability testing revealed that of the total fertile cyst, 40(54.05%) were viable and 34(45.95%) were non-viable (Table 4).

Table 4: Distribution of cyst in lung and liver in cattle based on fertility and viability

Organ	Sterility testing		Viability testing		
	Sterile	Fertile	Calcified	Viable	Non-viable
Lung	93(65.5%)	44(31%)	5(3.5%)	28(63.6%)	16(36.4%)
Liver	27(540.9%)	30(45.5%)	9(13.6%)	12(40%)	18(60%)
Total	120(57.7%)	74(35.6%)	14(6.7%)	40(54.1%)	34(45.9%)

Financial loss assessment

The direct financial loss was analyzed based on the information obtained during post mortem examination and interview. The 210 infected organ cattle imposed an estimated total loss of 17,844.90 ETB in the study abattoir during the study period. Accordingly, the annual loss due to organ condemnation was calculated to be 894,505.612 ETB (Table 5).

Table 5: Number of condemned, percent involvement and average unit price of organs in cattle during the study period

Organs inspected	No of organs condemned	Percent involvement of organ	Average unit price
Lung	173	43.3%	15
Liver	102	25.5%	146.7
Kidney	3	0.8%	75.4
Heart	1	0.3%	39.3
Spleen	3	0.8%	7
Total	282	70.5%	283.4

Discussion

The overall prevalence of hydatidosis in cattle slaughtered in Adama municipal abattoir during the study period was 52.5%. The current finding is in close agreement with that reported 52.69% in Hawassa (Regassa *et al*., 2010), 53.5% in Assela (Ararso, 1997), and 54.8% in Arsi region (Alemayahu, 1990). The present finding was higher than the previous works reported from in different part of the country ranging from 11.26% to 48.9% (Berhe, 2009; Kebede *et al*., 2009a; Kebede *et al*., 2009b; Terefe *et al*., 2012; Bekele *et al*., 2013; Adane and Guadu, 2014; Nasr and Mahendra, 2016). However, the current finding is lower than prevalence study in other areas like 57.6% in Assela (Gadisa and Addis, 2016), and 62.96% around Bale (Polydorou, 1981). These discrepancies in disease prevalence among the various studies in different areas might be due to the difference in availability and frequency of exposure of the final hosts among the infected intermediate hosts and vice-versa.

In the present study the variables such as, sex, age, breed, body condition showed statistical significant ($p< 0.05$) association whereas origin of the animals was statistically insignificant ($p>0.05$) with the prevalence of hydatidosis during the study period**.** In the present study male animals were highly affected (56.7%) than females (30.8%). This difference in the infection rate could mainly be due to the fact that most females came from the farm (intensive) which decreases the contact rate with the definitive host and contaminated pasture. However, the current finding disagrees with what was reported from Harar (Lemma *et al.,* 2014) where infection rate was high in female (14.5%) than

male (9.1%). With regard to age the infection rate was higher in cattle having age older than 5 years (66.1%) than in cattle having age ≤ 5years (29.5%). This finding was in agreement with the study in Gonder ELFORA abattoir carried out by Adane and Guadu (2014). This might be due to longer exposure time to *E. grannulosus* eggs in addition to weaker immunity against the infection in older animals. In addition, most of the older animals slaughtered were culled animals due to less productivity and hence were exposed to the disease over long period with an increased possibility of acquiring the infections.

In the current study the infection rate was higher in local (59.6%) than exotic/cross breed (24.7%) cattle. This finding disagreed with the finding in Harar (Lemma *et al.,* 2014). The lower infection rate in the present study might be due to the reason that most of the exotic/cross breeds came to the study abattoir from the intensive type of farming system contributing to lower chance of contact with the definitive host. In the present study, the infection rate of bovine hydatidosis was higher in animals having poor body condition than medium and in medium than in good body conditioned animals. This finding supported the study in Gonder by Adane and Guadu (2014). With respect to the origin of the slaughtered animals, though the result indicates that prevalence of the disease was higher in Kereyo and lowest in Wolenchite, it was statistically insignificant (Table1). This may be due to difficulty in getting exact origin of the animals and exchanging of the animals in local markets. This result is inconsistent with the result of Yetnayet (2010), who reported that there is significant association between bovine hydatidosis and animal origin which could be due to difference in social activity, attitude to dogs and climate in different regions.

In the present study the highest infection rate occurred in the lung only followed by lung and liver than other organs examined during the study period. This finding disagrees with the study in Dire Dawa (Mulatu *et al*., 2013). But it agrees with the study in Jimma municipal abattoir (Tolosa *et al.,* 2009) and in Sebeta (Mekuria and Hirpassa, 2015). This high infection rate in lung may be due to the possibility of the hexacanth embryo to enter the lymphatic circulation and be carried via the thoracic duct to the lung in such a way that the lung may be infected before/instead of the liver (Zewdu *et al.,* 2010). On the other hand, the higher infection rate in lung and liver than the other organ may be due to the reason that liver and lung possess greater capillaries that act as partial barrier for the ingested hexachant/onchospher embryos taking the portal

vein route and primarily affect the hepatic and pulmonary system sequentially before any other peripheral organ invasion (Esatgil and Tuzer, 2007).

In the present study, lung has higher number of cyst than liver; and liver than the other organs. This finding is nearly close to the findings of Fikre (1994) and Nebiyou (1990). Such variation in cyst abundances in an organ is explained as probably to the special distribution and infectivity of *Echinococcus* eggs (Gemmel, 1979). In connection with the cyst size, the proportion of small, medium and large cyst is higher in lung than liver (Table4). This finding was in line with the result of study in Kombolcha ELFORA abattoir (Abunna *et al*., 2012) and in Dessie (Melaku *et al*., 2012). Higher proportion of medium and large cysts in lung might be due to the thoracic duct through which the oncosphers reach the lung before the liver via entering the lymphatic system and the softness of the lung tissue that enable the oncosphers to easily grow to larger hydatide cyst. On the other hand, higher proportion of small cyst than medium and large might be the result of late infection due to heavy rain fall and continuous grazing in the past rainy season or due to immunological response of the hosts which may inhibit expansion of cyst size (Melaku *et al*., 2012). But the present study disagrees with the result of the study in Dire Dawa (Mulatu *et al*., 2013) whose finding showed that higher proportion of cysts was found in liver than in lung. Liver in the present finding has higher number of calcified cysts 74/94(78.7%) than the lung 20/94(21.3%). This might be due to the relatively higher number of reticulo-endothelial and connective tissue reaction of the organ (Adane and Guadu, 2014).

Among cysts tested for fertility, the highest percent of cyst detected were fertile cysts followed by sterile cysts and calcified cysts. Lung was found to harbor higher percent of fertile cyst than sterile and calcified cysts, while liver also hosted the highest percent of sterile (Table: 5). This finding disagrees with the report of Mulatu *et al*. (2013) in Dire Dawa who found 772/968 (79.75%) fertile,168/968(17.35 %) sterile cysts. Likewise, Mohammed et al (2014) in Dire Dawa reported that 92(58.88%) and 33(21.15%) were fertile and sterile, respectively; while Lemma et al (2014) in study carried out in Harar showed that high proportion of cysts were fertile. This difference in fertility rate between different organs might be due to the difference in the effect of drug on the parasite among different organ in different geographical area. The viability test revealed that out of the 74 cysts, liver has 12(40%) and 18(60%) while lung has 28(63.6%) and 16(36.4%) viable and non-viable cysts, respectively. Therefore,

more of lung's fertile cysts were viable but more of liver's fertile cysts were non-viable. This variation might be due to the difference between tissue resistances of organs for the growth of the cyst. This also might be due to the difference in action of drugs in the two organs. This finding agrees with the study in Debra Tabor Municipal Abattoir (Tadesse *et al.,* 2016).

The direct financial loss in Adama municipal abattoir due to organ condemnation during the study period was 17844.90 ETB and the annual financial loss was estimated to be 894505.612 ETB. This finding was much greater than the finding in Dire Dawa (Mulatu *et al*., 2013) (165876 ETB), Nekemte (Moje and Degefa, 2014) (8561.61 ETB) and Harar (Lemma *et al.,* 2014) (96315.00 ETB). The figure for the financial loss due to organ condemnation in the present study is very high for the country that goes through economic transformation plan.

Conclusion

In conclusion, the present study showed that the prevalence and the estimated direct financial loss due to organ condemnation of bovine hydatidosis were high. It was also evident that lung and liver were the two organs with the highest cyst distribution compared to other organs which potentially leads to higher rate of condemnation of these organs. Therefore, control of the parasite directed both in the intermediate and definitive host should be carried out. Additionally, aggressive meat inspection, proper disposal of affected organs and deworming of dogs in the study area should be carried out.

Acknowledgements

The authors would like to thank the meat inspectors at Adama Municipal Abattoir and laboratory workers at the Adama woreda parasitology laboratory who rendered technical assistance during the study.

Conflict of interest

There is no conflict of interest to declare

References

Abunna, F., Fentaye, S., Megersa, B., Regassa, A.,2012. Prevalence of bovine hydatidosis in Kombolcha ELFORA abattoir, North Eastern Ethiopia. *Open J. Ani. Sci.,*2(4), 281-286. 0913386721. http://dx.doi.org/10.4236/ojas.2012.24038.

Adane, M., and Guadu, T., 2014. Bovine Hydatidosis: Occurrence, Economic and Public Health Importance in Gondar ELFORA Abattoir. *Euro. J. App. Sci.,*6 (2), 11-19. DOI: 10.5829/idosi.ejas.2014.6.2.8585.

Alemayehu, L., 1990. Prevalence of hydatidosis in cattle, sheep, goats and Echinococcosis in Dogs in Arsi. DVM thesis, Faculty of Veterinary Medicine, Debrezeit Ethiopia. Pp. 26.

Ararso, O. (1997): The prevalence of hydatidosis in and around Assela. DVM Thesis, Faculty of Veterinary Medicine Addis Ababa University, Debrezeit, Ethiopia.

Azlaf, R. and Dakkak, A., 2006. Epidemiological study of the cystic echinococcosis in Morocco. *Vet. Parasitol.,* 137, 83-93.

Bekele, J., Kebede, W., Shimelis, S., Shiferaw, D.,2013. Prevalence and financial loss estimation of cystic echinoccocosis in cattle slaughtered at Mizan Teferi and Teppi municipal abattoir south western Ethiopia. *Euro. J. App. Sci.,*5(1), 12-18. DOI: 10.5829/idosi.ejas.2013.5.1.66102.

Berhe, G., 2009. Abattoir survey on cattle hydatidosis in Tigray region of Ethiopia. *Trop. Anim. Hlth. Prod.*, 41, 1347-1352. doi: 10.1007/s11250-009-9320-0.

Budke, M., Deplazes, P., and Torgerson, R., 2006. Global socioeconomic impact of cystic echinococcosis. *Emerg. Inf. Dis.,*12(2)**,** 296-303. doi: 10.3201/eid1202.050499.

CSA (2010). Central Statistical Agency Federal Republic Democratic of Ethiopia Agricultural Sample Survey 2008/2009(2001 E.C). Report on livestock and livestock characteristics.

Dalimi, A., Motamedi, G., Hosseini, M., Mohammadian, B., Malaki, H., Ghamari, Z. and Ghaffari, F.F., 2002. Echinococcosis/Hydatidosis in western Iraq. *Vet. Parasitol.,* 105(2), 161-171. doi.org/10.1016/S0304-4017(02)00005-5.

Denbarga, Y., Demewez, G., Sheferaw, D., 2011. Major Causes of Organ condemnation and financial significance of cattle slaughtered at Gondar Elfora Abattoir, Northern Ethiopia. *Global Veterinaria*, 7, 487-490.

Eckert, J., Schantz, P.M. and Gasser, B., 2002. WHO/OIE manual in echinococcosis in humans' animals. Geographic distribution and prevalence. World health Organization and World Organization for Animal Health, Paris, Pp. 101-143.

Elmahdi, I., Ali, E., Magzoub, Q., Ibrahim, M., Saad, A. and Roming, M., 2004. Cystic echinococcosis of livestock and human in central Sudan. *Ann. Trop. Med. Parasitol.,* 98, 473-479.

Esatgil, M.U and Tuzer, E., 2007. Prevalence of hydatidosis in slaughtered animals in Thrace, Turkey. *Turkiye Parazitol. Derg.*, 31(1). 41-45.

FAO, 2003. Diagnostic Manual on Meat Inspection for Developing Countries, Pp. 12-38.

Fikre, L., 1994. Hydatidosis in Nekemete prevalence in slaughtered cattle sheep, estimate economic loss and incidence in stray dogs. DVM thesis, Faculty of Veterinary Medicine, Debrezeit Ethiopia.

Gadisa, B., and Addis, M., 2016. The Abattoir Prevalence and Monetary Loss of Fasciolosis and Hydatidosis among apparently Healthy Slaughtered Cattle at Asella Town, Ethiopia, *World Appl. Sci. J.,* 34 (7): 897-904. DOI: 10.5829/idosi.wasj.2016.34.7.104144.

Gemmel, M.A.,1979. Hydatidosis control- A global view, *Aus. Vet. J.,* 55. 118-125. https://doi.org/10.1111/j.1751-0813.1979.tb15246.x.

Getachew, D., Almaw, G., and Terefe, G., 2012. Occurrence and fertility rates of hydatid cysts in sheep and goats slaughtered at Modjo Luna Export Slaughter House, Ethiopia. *Ethiop.Vet. J.,*16**,** 83-91. http://dx.doi.org/10.4314/evj.v16i1.7.

Getaw, A., Beyene, D., Ayana, D., Megersa, B., Abunna, F., 2010. Hydatidosis: prevalence and its economic importance in ruminants slaughtered at Adama municipal abattoir, central Oromia, Ethiopia. *Acta Trop..,* 113**,** 221–225. https://doi.org/10.1016/j.actatropica.2009.10.019.

Hossein, M., Ebrahim, S., Dezaki, F., Kheirandish, B., Ezatpour, S., Jahanbakhsh, M., Fasihi, H. 2014. Scolicidal Effects of Black Cumin Seed (*Nigella sativa*) Essential Oil on Hydatid Cysts. *Korean J. Parasitol.,*52(6), 653-659. doi: 10.3347/kjp.2014.52.6.653

Kebede, N., Tilahun, G. and Hailu, A., 2009a. Current status of bovine cysticercosis of slaughtered cattle in Addis Ababa Abattoir, Ethiopia. *Trop. Anim. Hlth. Prod.,*41(3), 290-294. DOI 10.1007/s11250-008-9188-4.

Kebede, N., Mitiku, A., Tilahun, G., 2009b. Hydatidosis of slaughtered animals in Bahir Dar abattoir, northwestern Ethiopia. *Trop. Anim. Hlth. Prod.,*41(1), 43–50. doi: 10.1007/s11250-008-9152-3.

Kebede W., Hagos, A., Girma, Z. and Lobago, F. 2009c. Echinococcosis/hydatidosis: its prevalence, economic and public health significance in Tigray region, North Ethiopia. *Trop.Anim. Hlth. Prod.,*41(6), 865-871. doi: 10.1007/s11250-008-9264-9.

Kebede, N., Abuhay, A., Tilahun, G. and Wossene, A., 2009d. Financial loss estimation, prevalence and characterization of hydatidosis of cattle slaughtered at DebreMarkos municipality abattoir, Ethiopia. *Trop.Anim. Hlth. Prod.,*41(8), 1787–1789. doi: 10.1007/s11250-009-9356-1.

Khadidja, H., Yahia, A., Ghadiri, Y., Vasile, C., 2011. Epidemiologic study of hydatidosis in the steppe regions of Djelfa, Algeria. *Sci.Parasitol.,*12(4),177-183.

Lemma, B., Abera, T., Urga, B., Niguse, A., and Agonafir, A., 2014. Prevalence of Bovine Hydatidosis and Its Economic Significance in Harar Municipality Abattoir, Eastern Ethiopia. *Amer.-Eurasian J. Scientific Res.,*9 (5), 143-149. DOI: 10.5829/idosi.aejsr.2014.9.5.9144.

Mekuria, S., and Hirpassa, L.,2015. Prevalence and Viability of Hydatidosis in Cattle Slaughtered at Sebeta Municipal Abattoir, Central Ethiopia. *Acta Parasitol. Globalis,*6 (1), 01-07. DOI: 10.5829/idosi.apg.2015.6.1.9112.

Melaku, A., Lukas, B. and Bogale, B., 2012. Cyst Viability, Organ Distribution and Financial Losses due to Hydatidosis in Cattle Slaughtered at Dessie Municipal Abattoir, North-eastern Ethiopia, *Vet. World*, 5(4), 213-218.

Mohammed, A., Chanie, M., and Warsame, I., 2014. Prevalence, Economic and Public Health Significance of Camel Hydatidosis in Dire Dawa Municipal Abattoir, Eastern Ethiopia. *Acta Parasitol. Globalis.* 5 (2): 98-106. DOI: 10.5829/idosi.apg.2014.5.2.84209.

Moje, N., and Degefa, A.,2014. Prevalence, cyst viability, organ distribution and financial losses due to hydatidosis in cattle slaughtered at Nekemte municipal abattoir, western Ethiopia. *J.Vet. Med.Anim. Hlth.,*6(11), 280-288. DOI: 10.5897/JVMAH2014.0316.

Mulatu, M., Mekonnen, B., Tassew, H., Kumar, A., 2013. Bovine Hydatidosis in Eastern Part of Ethiopia, *Momona Ethiop. J. Sci.*, 5(1),107-114.

Nasr, W., and Mahendra, P., 2016. Prevalence, cyst viability, fertility and economic significance of bovine hydatidosis in an abattoir at Kombolcha, Ethiopia. *Haryana Vet.,*55 (1), 17-22.

Nebiyou, G., 1990. Hydatidosis/Echinococcosis in cattle slaughtered at Bahir-dar Municipality slaughter house. DVM thesis, Faculty of Veterinary Medicine, Debrezeit, Ethiopia.

NMSA., 2016. National Meteorology Service Agency, 2015/2016, Addis Ababa, Ethiopia.

Nicholson, M. and Buttrworth, M., 1986. A guide condition scoring of Zebu cattle international livestock center for Africa (ILCA), Addis Ababa, Ethiopia, p 3.

Ogunrinade, F. and Ogunrinade, B., 1980. Economic importance of bovine fascilosis in Nigeria. *Trop. Anim. Hlth. Prod.,* 12(3), 155-160.

Oostburg, B.F., Vrede, M.A., and Bergen, A.E., 2000. The occurrence of polycystic echinococcosis in Suriname. *Ann. Trop. Med. Parasitol*., 94(3), 247-252. DOI: 10.1080/00034980050006429.

Polydorou, K., 1981. Animal health and economics. Case study: Echinococcosis with a reference to Cyprus (Dog). Bull In. Epis. 93(5-3),981-992.

Regassa, F., Molla, A. and Bekele, J., 2010. Study on the prevalence of cystic hydatidosis and its economic significance in cattle slaughtered at Hawassa municipal abattoir. *Trop. Anim. Hlth. Prod.,*42(5)**,** 977–984. doi: 10.1007/s11250-009-9517-2.

Reshid, K., 2017. A city profile performer with municipal service office of Adama unpublished document, Pp. 14-16.

Rostami, N., Nazemal, M., Hosseini, M., Fasihi, E. and Harandi, M., 2010. Echinococcosis: based on molecular studie s in Iran. *Gastroenterol. Hepatol. Bed Bench*, 3,169–176.

Sadjjadi, S.M., 2006. Present situation of Echinococcosis in the Middle East and Arabic North Africa. *Parasitol. Int*., 55**,** 197– 202.

SPSS., 2016. SPSS 1.0 User's Guide. USA: SPSS Inc.

Tadesse, A., Ayele, B., Asefa, A., and Haile, B., 2016. Prevalence and Economic Significance of Bovine Cystic Echinococcosis in Debra Tabor Municipal Abattoir, North West Ethiopia. *Acta Parasitol. Globalis,***7** (3), 114-120. DOI: 10.5829/idosi.apg.2016.7.3.10536.

Terefe, D., Kebede, K., Beyond, D., and Wondimu, A., 2012. Prevalence and financial loss estimation of hydatidosis of cattle slaughtered at Addis Ababa abattoirs enterprise. *J.Vet. Med. Anim.Hlth.,*4(3), 42-47.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd ed.y Blackwell Science Ltd, a Blackwell Publishing company, Pp. 233-236.

Tolosa, T., Tigre, W., Teka, G. and Dorny, P., 2009. Prevalence of bovine cysticercosis and hydatidosis in Jimma municipal abattoir, SouthWest Ethiopia. *Onderstepoort J. Vet. Res.,* 76(3), 323–326.

Yemane, G. 1990. Preliminary study of echinococcosis in ruminants slaughtere at Adama Abattoir. DVM thesis, Faculty of Veterinary Medicine, Debrezeit, Ethiopia.

Yetnayet, G.,2010. Prevalence and economic significance of Bovine hydatidosis in cattle slaughtered at Gondar ELFORA abattoir, North Gondar. DVM thesis, Faculty of Veterinary Medicine, University of Gondar, Gondar, Ethiopia.

Zewdu, E., Teshome, T., Makwoya, A.,2010. Bovine Hydatidosis in Ambo Municipality Abattoir, West Shoa, Ethiopia. Ethiopia. *Ethiop. Vet. J.,*14 (1), 1-142.

Comparative diagnostic techniques in ruminant Fasciolosis: fecal sedimentation, indirect ELISA, liver inspection and serum enzyme activities

Meskerem Adamu[1*], Abebe Wossene[2], Getachew Tilahun[3] and Asoke Kumar Basu[2]

[1]*Ethiopian Biotechnology Institute, Addis Ababa, Ethiopia*

[2]*Department of Pathology and Parasitology, Collage of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia*

[3]*Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia*

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

Abstract

Different diagnostic tests have some disadvantages in diagnosing Fasciolosis. A cross-sectional study was conducted to estimate the prevalence and to compare the different diagnostic techniques in ruminant Fasciolosis: ELISA, biochemical analysis, liver and coprological examination. Out of the 324 cattle, 350 sheep and 385 goats slaughtered at Bishoftu, ELFORA Export Abattoir, 125 (38.5%), 4 (1.1%) and 2 (0.5%) and 108 (33%) 4 (1.1%) and 2 (0.5%) were found to be positive for lesions of fasciolosis and Fasciola eggs, respectively. The results of different diagnostic techniques in randomly selected 134 cattle, 68 sheep and 22 goats samples were compared. Out of the 134 examined cattle, 100 (75%) were found to be positive for antibodies against Fasciola specific f2 antigens followed by 54 (41%) for lesions of Fasciolosis, 40 (30%) for Fasciola eggs and 56 (42%) and 100 (75%) of the samples had an increased level of serum enzyme GGT and LDH above the normal values, respectively. The overall assessment indicates the test agreement of ELISA findings with the results of fecal examination is weak (Kappa=0.236), ELISA findings with the results of liver lesions is moderate (Kappa=0.373), liver lesions with GGT determination is moderate (Kappa=0.332) and liver lesions with level of LDH is absent (Kappa=-0.066). However, there was a strong relationship (Kappa=0.758) between fecal examination and liver lesions. In small ruminants, the prevalence of Fasciolosis was lower both in coproscopy and indirect ELISA tests: only one out of 68 sheep was positive coproscopically and 3 were positive serologically; and one out of 22 goats tested was positive both at coproscopy and serological examinations. The sensitivity of ELISA was higher compared to the oth-

ers techniques in diagnosing ruminant fasciolosis. Coprological examination should be repeated and supported by other diagnostic methods.

Key Words: Coproscopy; ELISA; *Fasciola*; Liver enzymes; Ruminants

Introduction

The economic losses due to fasciolosis throughout the world are enormous. In Ethiopia, the prevalence of bovine fasciolosis has been reported to exist in many parts of the country (Abera, 1990; Zewdu, 1991; Abdul, 1992; Rahmeto, 1992). *Fasciola hepatica* and *F. gigantica* are causing major economic losses in livestock in East Africa (Malone *et al.,* 1998). *F. hepatica* was shown to be the most important fluke species in Ethiopian livestock with distribution over three quarter of the nation except in the arid north- east and east of the county while the distribution of *F. gigantica* was mainly localized in the western humid zone of the country that encompasses approximately one fourth of the nation (Yilma and Malone, 1998). Diagnosis of Fasciolosis is based on clinical signs, seasonal occurrence, liver and coprological examination, which may be supplemented by two other laboratory tests. The first estimation of plasma levels of enzymes released by damaged liver cells and the second is the detection of antibodies against components of flukes, the ELISA and passive haemoagglutination test being the most reliable (Urquhart *et al.,* 1996). Proper diagnosis of the disease is important in prescribing effective drugs and assists any control programs directed to Fasciolosis. However, the different diagnostic techniques used today to confirm Fasciolosis in ruminants have their limitations. Coprological analysis is still commonly employed to diagnose bovine Fasciolosis despite the overwhelming consensus that this method is not wholly reliable. On the other hand, since weak infections cannot be detected, the animal become the source of new infection (Sanchez-Andrade *et al.,* 1995). The most direct and reliable technique for the diagnosis of Fasciolosis is liver examination at slaughter or necropsy. But using this diagnostic technique it is impossible to detect Fasciolosis in live animals. Serum activities of lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) may be used as markers of the different stages of *Fasciola* infection in sheep, indicating the presence of cell necrosis caused by juvenile migrating flukes and bile duct lesions associated with mature helminthes, respectively (Ferre *et al.,* 1997). Different immunodiagnostic tests have been used in the early immune diagnosis of Fasciolosis, but they have some disadvantages, such as cross-reactions with other trematodes, leading to false positive results (Gonenc *et al.,* 2004).

The aim of this study was, therefore, to compare the results of different diagnostic techniques for Fasciolosis in slaughtered ruminants at ELFORA Export Abattoir, Bishoftu and to assess the extent of correlation of pathological lesion caused by flukes with the results of enzyme analysis.

Material and methods

Study area and study population

Data were collected from ELFORA Export Abattoir found in Bishoftu. Study animals were ruminants (cattle, sheep and goats). Animals were purchased from different parts of the country and brought to the abattoir. According to the information taken from the abattoir, sheep and goats were purchased from the arid eastern part of the country. Slaughtered animals were usually between 1 and 4 years of age for sheep and goats and 1 and 10 years of age for cattle.

Study methodology

Study design and sampling method:

To estimate the prevalence of the disease a cross-sectional study was conducted. The desired sample size for the study was calculated using Win-Episcope 2,0 computer software program Thrusfield (1995), with 95% confidence interval and at 5% absolute Precision. Assuming 42.9% average prevalence of Fasciolosis for cattle, 50% for sheep and goats in ELFORA Export Abattoir, a total of 1059 ruminants: 324 cattle, 350 sheep and 385 goats were examined. Data were collected from all study animals for liver and coprological examination and because of the testing capacity of the ELISA kit, we compared the diagnosis results of randomly selected 134 cattle, 68 sheep and 22 goats samples. Each animal was diagnosed against *Fasciola* infection using serological tests (indirect ELISA), biochemical analysis (serum enzyme GGT and LDH measurements) as well as liver and coprological examination. Animals were tagged on one ear. Blood and fecal samples were collected one day before slaughter. On the next day data was collected from the liver of corresponding ear tagged animal after slaughter.

Fecal egg examination:

For coprological examination fecal samples were collected from the study animals directly from the rectum. The collected samples were taken to the

laboratory with tightly closed universal bottles and examined for *Fasciola* eggs by using simple sedimentation McMaster method as described by Antonia *et al* (2002).

Postmortem liver examination:

The liver of each animal was removed and examined for gross pathological lesions, the bile duct and the gall bladder were incised and then the organ was cut to detect immature and adult flukes and to identify the fluke species. Identification of the fluke species involved was carried out by using different parameters such as flukes, cone and shoulder size, shoulder shape and flukes color (Soulsby, 1982). The liver lesion in the acute Fasciolosis is hypertophic and haemeorrhagic, on careful examination, young flukes are found throughout the organ, the peritoneal cavity is infiltrated with a serohemorrhagic fluid and hemorrhagic patches on the peritoneum. In the chronic Fasciolosis the essential lesion is a progressive biliary cirrhosis, hard fibrotic liver in which the bile ducts are prominent, thickened, fibrous and in cattle often calcified and cholangitis with dilated bile ducts containing flukes and their eggs. Eenlargements of the gallbladder and aberrant migration of the flukes is more common in cattle and encapsulated parasites are often seen in the lungs (Soulsby, 1982; Troncy, 1989; Urquhart *et al*., 1996). The level of pathological lesions within the herd were categorized based on the approach by Ogunrinade and Ogunrinade (1980) as follows; (a) lightly affected if small portion of the organ is affected and only one bile duct is enlarged on the visceral surface of the liver; (b) moderately affected if half of the organ is affected and two or more bile ducts are enlarged and (c) severely affected if most portion of the organ is involved and the liver is cirrhotic.

Liver enzyme assay:

For enzyme analysis, blood samples were collected using sterile plain vacutainers from the jugular vein of the study animals and the serum separated and kept at-20 ^{o}C until tested. Serum enzyme activities of LDH and GGT were measured using commercial kits and spectrophotometer according to the kinetic method described by Kerr (2002), in which working reagents were diluted with samples and controls and were preincubated, the photometer was adjusted to 0 absorbance for absorbance reading and for calculating the mean average change in absorbance per minute or the activity of an enzyme analyte. The mean data were interpreted according to the reference interval values indicated for cattle, sheep and goats as 4.9–26, 20–44 and 20–50 for serum

enzymes GGT and as 309–938, 83–476 and 79–265 for serum enzymes LDH, respectively (Merck, 2006)

Serology for antibody analysis:

Serum was tested for the presence of *Fasciola* antibodies against the specific "f2" antigens using *Fasciola* antigen coated ELISA diagnostic kit as described by Rickard (1995) and Cervi *et al* (1996). Negative and positive sera were tested in duplicate on antigen pre-coated polystyrene microplates with a total of 45 samples per plate (Institut Pourquier). Controls and serum samples were diluted 1:20 in the ELISA buffer on antigen pre-coated polystyrene micro plates, plates were washed with wash solution 3 times, diluted with the conjugate (an anti-bovine immunoglobulins monoclonal coupled to peroxydase), after a three times washing steps the substrate (TMB) was added to the conjugate, plats were incubated for 20 minutes at 21°C away from the light forming a blue compound becoming yellow after blocking. The intensity of the color was a function of the rate of antibodies present in the serum sample. The different classes of seropositivity were determined according to the results obtained from the control sera provided in the kit. The test result was considered reliable when the positive control serum has a minimum uncorrected OD 450 value of 0.350 and a ratio between the corrected OD 450 value of the positive control and uncorrected OD 450 value of the negative control is greater than or equal to 3.5. Reading and interpretation of the corrected optical densities (OD) 450 for each serum sample was done by subtracting the OD 450 value obtained from uncoated well from the OD450 from a coated well and the level of infestation within the herd were determined as listed in the table below (Table 1).

Table 1. Enzyme Linked Immunosorbent Assay test validation and interpretation

S/P % of the sample Infestation within the herd	**results and the level of correlation between test**
%E/P > 150%	+++=Strong Infestation (>50% of infestation)
S/P included between 80% and 150%	++=Medium Infestation (between 20% and 50% of infestation)
30<%E/P<80%	+=Low Infestation (<20% of infestation)
S/P<30%	0=No infestation

S/P (Seropositivity) = corrected OD450 value of the sample / corrected OD450 value of the positive control x 100.

Source: Institute Pourquier

Data analysis:

Data was analyzed using Microsoft Excel spreadsheet for data storage and descriptive statistics. Results of diagnostic techniques were analyzed using WinEpiscope 2.0 software program (2000), testes such as confidence interval and the kappa statistic (agreement among different tests), sensitivity and specificity. Agreement between tests was compared using kappa statistic where the kappa value 0.8-1.0 denotes very good, 0.6-0.8 sustained and 0.4--0.6 moderate (Thrusfield, 1995).

Results

Coprological study:

Out of the 324 cattle, 350 sheep and 385 goats slaughtered at ELFORA Export Abattoir, 33%, 1.14% and 0.51% were positive for *Fasciola* eggs in cattle sheep and goats, respectively (Table 2). Out of the 134 examined cattle 30% were positive for *Fasciola* eggs (Figure 4).

Table 2. Prevalence of Fasciolosis in different species of ruminants slaughtered at ELFORA Export Abattoir based on coproscopy.

Ruminants	Sample size	Prevalence	95% ci
Cattle	324	108(33%)	28.6-38
Sheep	350	4(1.14%)	0.4-3
Goat	385	2(0.5%)	0.1-2

Postmortem inspection:

Out of the 324 cattle, 350 sheep and 385 goats slaughtered at ELFORA Export Abattoir, 38.5%, 1.14% and 0.51%, were found to be positive for lesions of Fasciolosis during postmortem examination, respectively (Table 3). Out of the 134 examined cattle 41% were positive for lesions of Fasciolosis (Figure 2).

Table 3. Prevalence of Fasciolosis in different species of ruminants slaughtered at ELFORA Export Abattoir based on postmortem inspection.

Ruminants	Sample size	Prevalence	95% ci
Cattle	324	125 (38.5%)	33.6-44.3
Sheep	350	4(1.14%)	0.4-3
Goat	385	2(0.5%)	0.1-2

Based on the classification of liver pathology, from the 54 out of 134 infected cattle livers, 31(23%) of the livers were lightly infected, 20(15%) moderately infected and 3(2.2%) were severely damaged (Figure 1).

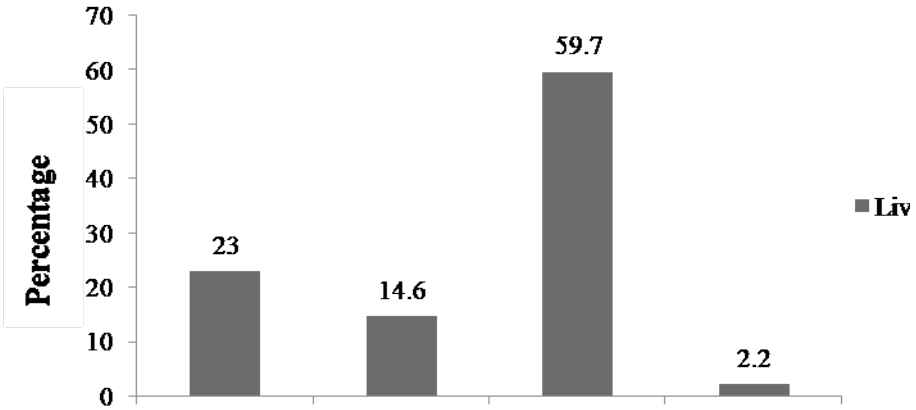


Figure 1. Classification of liver pathology

Serum enzyme analysis: Out of 134 examined cattle for of GGT and LDH, 42% and 75% of the samples had an increased level of GGT and LDH, above the normal values, respectively (Figure 2).

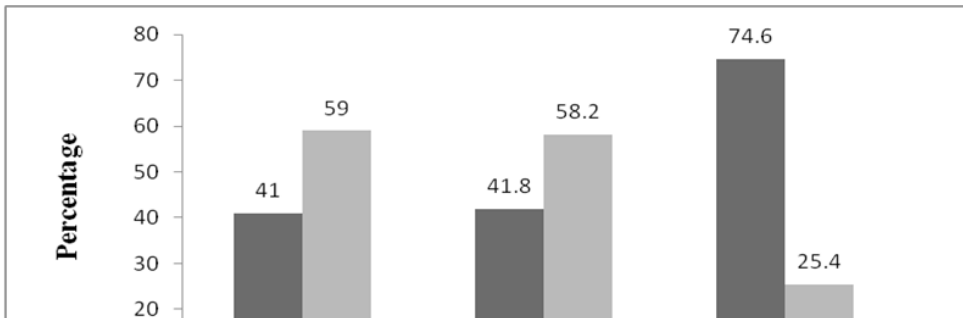


Figure 2. Comparison of enzyme activities and liver pathology in cattle

Serological examination (indirect ELISA):

Out of the 134 cattle serum analyzed, 75% of the samples were found to be positive for antibodies against *Fasciola* specific f2 antigens. Based on antibody detection ELISA against *Fasciola* specific f2 antigens, the levels of infestation, from the 100 out of 134 antibody positive samples against *Fasciola* specific f2 antigens, 23% of the animals were lightly infected, 48% were moderately infected and 1.5% were severely infected (Figure 3 and 4). The sensitivity and specificity of ELISA were 100% and 42.5% respectively.

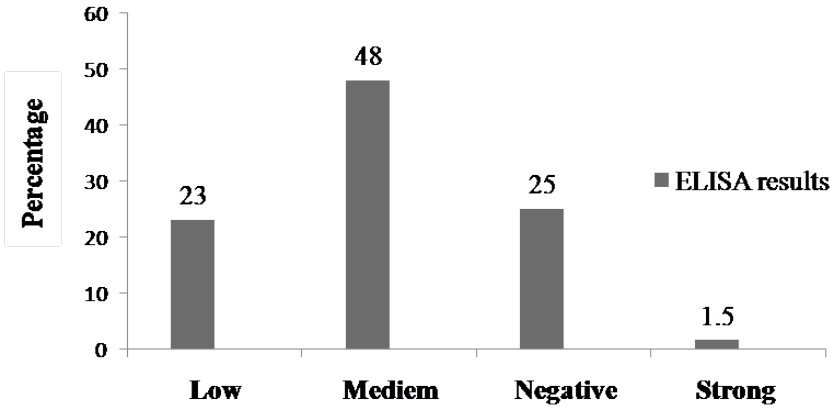


Figure 3. ELISA results and level of infestation within the herd

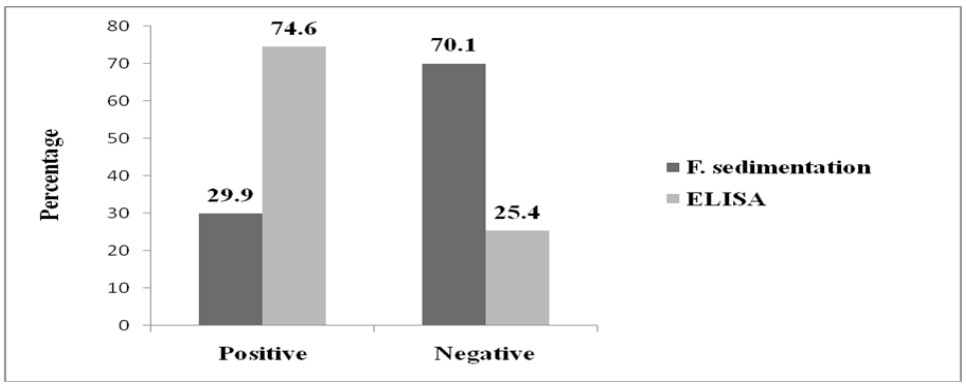


Figure 4. Comparison of ELISA and fecal sedimentation results

Comparative results of different diagnostic tests:

Summary results of the different diagnostic test employed on 134 cattle and the statistical analysis results are presented in (Table 4). There was a strong relationship between fecal examination and postmortem findings of liver lesions, with Kappa value, (Kappa= 0.758). Test agreement between postmortem findings of liver lesions and ELISA (Kappa=0.373) and between postmortem findings of liver lesions and ***s***erum enzyme activities of GGT (Kappa=0.332) is moderate, the relationship between fecal examination and ELISA (Kappa= 0.236) is weak and agreement between postmortem findings of liver lesions and ***s***erum enzyme activities of LDH is absent (Kappa= -0.066).

Table 4. Comparison of the different diagnostic methods in relation to the results of coproscopy and postmortem examination

Factor		ELISA		GGT		LDH		PMI	
		Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Fecal examination	Negative	33	60	63	29	21	71	79	14
	Positive	1	40	15	26	10	31	1	40
	Kappa test	0.236		0.291		-0.011		0.758	
Liver Examination (PMI)	Negative	34	46	57	22	16	63		
	Positive	0	54	21	33	15	39		
	Kappa test	0.373		0.332		-0.066			

Discussion

In the present finding, the overall prevalence of cattle Fasciolosis based on coproscopy (33%) and postmortem examination (38.5%) is comparable with the results of many authors in various parts of Ethiopia (Getu, 1987; Wondwosen, 1990; Rahmeto, 1992; Adem, 1994; Abduljebar, 1994; Hailu, 1995; Mesfin, 1999). The overall prevalence of small ruminant Fasciolosis, resulting from coproscopy and postmortem examination was 0.5% in goats and 1.14% in sheep. The present finding is by far lower than the abattoir survey report of Daniel (1995), who reported 14.8% of ovine Fasciolosis in Dire Dawa and Rahmeto (1992), who reported 12.4% of caprine Fasciolosis in Wolliso. The lower prevalence of Fasciolsis reported using coproscopy in bovine (30%) compared to post mortem finding (41%), indicates the less sensitivity of the test in de-

tecting the actual presence of the disease. According to Abduljebar (1994), the prevalence of Fasciolosis by coproscopy was 34% while 49% by post mortem examination; the sensitivity of sedimentation technique in detecting *Fasciola* infection is about 33% while Sisay and Nibret (2013), reported much better, 69.5% sensitivity. A longer period from 8-15 weeks after infection is needed for the appearance of *Fasciola* eggs in the feces (Hillyer, 1999; O'Neill *et al*., 2000; Cornelissena *et al*., 2001; Sanchez-Andrade *et al*., 2002). Furthermore, detection of *Fasciola* eggs and diagnosis of the disease in some cases is difficult during the patent period because eggs are expelled intermittently depending on the evacuation of the gall bladder and the biology of the *Fasciola* (Troncy, 1989). In the current study, the higher prevalence of Fasciolsis reported using indirect ELISA method in bovine (75%) compared to post mortem finding (41%), and to coproscopy (30%), indicates the high sensitivity of ELISA (100%) in diagnosing fasciolosis.

The overall assessment indicates the relatedness of ELISA findings with postmortem findings of liver lesions and fecal examination results. Our finding in cattle is in agreement with Hillyer *et al* (1996), who tested 147 cattle and found that 38 of the cattle were positive parasitologically while 84 were positive serologically with 82% sensitivity. The immunoenzymatic techniques such as indirect ELISA have been found to be very suitable for the diagnosis of Fasciolosis due to their high sensitivity and the possibility of processing many sera samples (Arriaga *et al.,* 1989). Martinez *et al* (1996) and Hillyer (1999), also indicated that these techniques are important for the diagnosis of fasciolosis because of an earlier detection than coproscopic is allowed. Ibarra *et al* (1998), were able to compare the sensitivity, specificity and usefulness of the DIG-ELISA, DOT-ELISA and indirect ELISA tests for determining the seroprevalence of Fasciolosis in cattle under tropical conditions in Mexico and they found that indirect ELISA yielded the highest level of IgG and *F. hepatica* antibodies with a sensitivity of 97% and specificity of 98.8% than Dig ELISA 97.5% and 80.0% and Dot ELISA 93.1% and 95.4%, respectively.

In the present study, Out of 80 cattle, which were negative for lesions of *Fasciola*, 46 of them had antibodies against F2 antigens of *F. hepatica*. Animals with no detectable liver lesions but positive for serology might have been at their early stage of invasion with immature flukes. However, the presence of antibodies does not always correlate to active Fasciolosis because antibody levels diminish slowly after cure and remains positive for 5 months after treatment with or without the presence of liver flukes (Hillyer, 1999; Sanchez-Andrade *et al.,*

2002). By means of the indirect ELISA, based on excretory, secretory antigen, it has been also possible to detect experimental *Fasciola* infections in cattle from the third to the fifth week after infection during the liver migratory phase of immature worms María Soledad (1992) on the other hand, a longer period is required to detected positive animals through fecal examination. Cornelissen *et al* (2001), were able to detect positive animals by egg count only from week 9 in sheep and even later in cattle from week 15 onwards after natural infection of animals with *F. hepatica,* and Arias *et al* (2005), in their experimental work have detected positive animals through fecal examination 11 weeks after primary infection. Perez *et al* (2005), reported that eggs in stools indicates the presence of mature adult flukes in the bile ducts and gall-bladder, but a period longer than 10 weeks after infection is required so most of the pathological damage has already occurred. The classification of liver pathology and the level of *Fasciola* infestation within the herd were compared based on postmortem findings of liver lesions and antibody detection by ELISA, the results of light and sever infection were similar, 23% and 2% of the animals were lightly and severely infected respectively, in both tests. However, 48% and 15% of the animals were moderately infected based on ELISA and liver lesions of *Fasciola*, respectively this indicates the high sensitivity of ELISA in diagnosing Fasciolosis.

Contrary to the findings obtained in cattle, the overall finding regarding Fasciolosis in small ruminants was low both through the standard fecal sedimentation techniques and ELISA. Out of the randomly selected 68 sheep only 1 was positive parasitologically and 3 were positive serologically and of 22 goats tested, 1 was positive parasitologically and serologically. These findings generally suggest the very low prevalence of Fasciolosis in small ruminants. This perhaps related to the origin of the animals. Almost all small ruminants slaughtered originate from the mid and lowland parts of the eastern Ethiopia, areas most of the time considered to be low risk areas for *F. hepatica* infection due to the arid or semiarid ecology. *Fasciola hepatica* was shown to be the most important fluke species in Ethiopian livestock with distribution over three-quarter of the nation except in the arid north-east and east of the county while the distribution of *F. gigantica* was mainly localized in the western humid zone of the country that encompasses approximately one fourth of the nation (Malone *et al*., 1998; Yilma & Malone, 1998).

Changes in serum enzymes are indicators of hepatic metabolism impairment (Galtier *et al.,* 1994; Ferre *et al.,* 1996). Certain tissue cells contain characteristic enzymes, which enter the blood only when the cells to which they are

confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage. The level of GGT rise dramatically with obstructive diseases of the biliary tract and liver cancers and is especially useful in assessing liver function associated with liver disease (Ramnic, 1999; Charles, 2003; Merck, 2006). In support of the above statement, our findings regarding the level of GGT that 42% of the samples had an increased level of serum enzyme GGT suggest its association with liver lesions resulted at postmortem examination on 41% of cattle. The test of agreement between the liver examination and GGT determination was however moderate. Out of the 54 cattle with known liver lesions of *Fasciola*, 33 of them were having elevated levels of GGT than normal value for cattle. As the same time, out of the 79 cattle without liver lesions of *Fasciola*, 22 of them were having elevated levels of GGT than normal value for cattle. This might indicate increased levels of GGT could be yielded due to other liver diseases with or without the presence of Fasciolosis. The level of LDH has shown to be generally elevated both in negative as well as positive animals for liver lesions indicating the absence of agreement between liver lesions and level of LDH. Seventy five percent of the examined cattle had elevated levels of LDH in their serum. LDH modification indicates mostly a cell necrosis during migration of young flukes through the liver parenchyma. LDH provide information on the passage of young flukes through the liver parenchyma (Charles, 2003), while GGT would be more associated with bile duct damages. GGT increase indicates penetration of the bile ducts by flukes, causing a hyperplastic cholangitis (Galtier *et al.,* 1986; Ferre *et al.,*1994; 1995a; 1995b; 1996; 1997). Lactic Dehydrogenase (LDH -5) is useful in assessing acute, ongoing liver disease and neither is usually increased in chronic liver disease. LDH-5 can also caused by hepatocellular disease, in addition none of the common tests (bilirubin, GGT, GLDH, AP, LDH, AST, or alanine transaminase) for liver damage or function are clinically useful for detection of hepatic disease when used alone (Ramnic, 1999; Merck, 2006). Serum concentrations of liver-specific enzymes are generally higher in acute liver disease than in chronic liver disease. They may be within normal limits in the later stages of sub acute or chronic hepatic disease (Merck, 2006). In addition, these enzymes are also produced by a variety of other tissue and organ cells and hence results should be interpreted in conjunction with other diagnostic tests.

In conclusion, coprological examination for parasite eggs has significant limitations in detecting Fasciolosis. Cattle, which were negative for liver lesions of *Fasciola* and for *Fasciola* eggs at coproscopy, were having antibodies against *Fasciola* specific antigens. ELISA method is a good diagnostic tool and may allow early detection of disease. Measurement of hepatic enzymes such as GGT

and LDH are supportive indicators in the diagnosis of Fasciolosis. There was a strong relationship in the diagnosis of Fasciolosis between fecal examination and findings of liver lesions. The test agreement between ELISA findings and the results of fecal examination is weak, the relationship between ELISA findings and examination of liver lesions and between ***s***erum enzyme GGT activities and the findings of liver lesions is moderate and the relationship between liver lesions and level of LDH is absent. Coprological examination should be repeated and supported by other diagnostic methods for better diagnostic techniques and to recommend appropriate drugs and timely intervention. Enzyme analysis needs to be done to know the degree of hepatic damage.

Acknowledgements

Authors would like to thank the Elfora export abattoir for providing data for the study purpose, Addis Ababa University, Faculty of Veterinary Medicine for financial support, The National Veterinary Institute and the technical staffs for their support in allowing us to use their laboratory. We thank the Institute of Pourquier, France for providing us the Fasciola ELISA kit for free of charge.

Conflict of interest

There is no conflict of interest to declare

References

Abdul, J. R., 1992. Economic significance of bovine fasciolosis and Hydatidiosis in Soddo. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM Thesis.

Abduljebar, M., 1994. Prevalence and economic significance of bovine Fasciolosis in Sinana District, Bale-Region. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM Thesis.

Abera, B., 1990. Prevalence and economic significance of Fasciolosis in "Neur cattle" slaughtered at Dembi–Dollo slaughter house. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM Thesis.

Adem, A., 1994. Prevalence of bovine and ovine Fasciolosis: A preliminary survey around Ziway Region (Shewa). Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM Thesis.

Antonia, M., Conceiçao, P., Rute, M., Durao, Isabel, H., Costa, Jose., Correia de Costa., 2002. Evaluation of a simple sedimentation method (modified MacMaster) for diagnosis of bovine Fasciolosis. *Vet. Parasitol.,* 105(4), 337-343. https://doi.org/10.1016/S0304-4017(02)00016-X.

Arias, M., Hillyer, G.V., Sanchez-Andrade, R., Suarez, J.L., Pedreira, J., Lomba, G., Diaz, P., Morrondo, P., Diez-Banos, P., Paz-Silva, A., 2005. A 2.9KDA *Fasciola hepatica* recombinant protein-based ELISA test for the detection of current ovine Fasciolosis trickle infected. *Vet. Parasitol.,* 137(1-2), 67-73. https://doi.org/10.1016/j.vetpar.2005.12.015.

Arriaga, de M. C., Paniagua, R. Ruiz., Navarrete, A., Bautista, C. R., Morilla, A., 1989. Comparison of dot Enzyme Linked Immunosorbent Assay (dot-elisa), passive haemaglutination test (pht) and thin layer immunoassay (tia) in the diagnosis of natural or experimental *F. hepatica* infections in sheep. *Vet. parasitol*., 30 (3), 197–203. https://doi.org/10.1016/0304-4017(89)90015-0.

Cervi, L., Rubinsteni, H and Masih, D. T., 1996. Involvement of excretion-secretion products from *Fasciola hepatica* inducing suppression of the cellular immune responses. Cordova, Argentina. *Vet. Parasito.,* 61(1-2), 97-111. https://doi.org/10.1016/0304-4017(95)00816-0.

Charles, E. Ophard., 2003. Diagnostic serum enzymes review. Elmhurst College. *Virtual Chembook*. http://www.hendrickhealth.org/healthy/000804.htm.

Cornelissena, Jan. B., Cor, W. J., Gaasenbeeka, P., Fred, P. H., Borgsteedea, H.M., Wicher, G., Holland, b.C., Michiel, M., Harmsena, Wim., Boersm, J.A., 2001. Early immunodiagnosis of fasciolosis in ruminants using recombinant *Fasciola hepatica* cathepsin L-like proteaseq. *Int. J. parasitol*., 31(7), 728-737. https://doi.org/10.1016/S0020-7519(01)00175-8.

Dagne, M., 1994. Survey on prevalence and economic significance of Bovine Fasciolosis in Debre Berhan region. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM thesis.

Daniel, F., 1995. Economic importance of organs condemnation due to Fasciolosis and Hydatidosis in cattle and sheep slaughtered at Dire Dawa Abattoir. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM thesis.

Ferre, I., Barrio, J.P., Gonzalez-Gallego, J., Rojo-Vazquez, F. A., 1994. Appetite depression in sheep experimentally infected with *Fasciola hepatica. Vet. Parasitol*., 55(1-2), 71-79. https://doi.org/10.1016/0304-4017(94)90056-6.

Ferre, I., Lopez, P., Gonzalo-Orden, M., Julian, M. D., Rojo, F. A., Gonzalez-Gallego, J., 1995a. The effects of subclinical fasciolosis on hepatic secretory function in sheep. *Parasitol. Res*., 81(2), 127-131.

Ferre, I., Lopez, P., Rojo-Vazquez, F., Gonzalez-Gallego, J., 1996. Experimental ovine fasciolosis: Antipyrine clearance as indicator of liver damage. *Vet. Parasitol*., 62 (1-2), 93-100. https://doi.org/10.1016/0304-4017(95)00857-8.

Ferre, I., Ortega-Mora, L.M., Rojo-Vazquez, F.A., 1995b. Seroprevalence of *Fasciola hepatica* infection in sheep in northwestern Spain. *Parasitol. Res*., 81(2), 137-142.

Ferre, I., Ortega-Mora, L., Rojo-Vazquez, F.A., 1997. Serum and Bile antibodies responses (IgG and IgA) during sub clinical *Fasciola hepatica* infection in sheep. *Vet. Parasitol.,* 68(3), 261-267. https://doi.org/10.1016/S0304-4017(96)01070-9.

Galtier, P. L. G., Tufenkji, A. E., Franc, M., 1986. Incidence of experimental fascioliasis, on the activitiy of drug metabolizing enzymes in lamb liver. *Drug metab. Dispos*., 14(1), 137-141.

Galtier, P., Coulet, M., Sutra, J.F. Biro., Souvour, B., Aluinerie, M., 1994. *Fasciola hepatica*: Mebendazole and Thiabendazole pharmacokinetics in sheep. *Parasitol*., 79(2),166-176. https://doi.org/10.1006/expr.1994.1076.

Getu, D., 1987. A study on the Incidence and Economic Significance of fasciolasis in Woliata Awraja. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM thesis.

Gonenc, B., Sarimehmetoglu, H.O., Kara, M., Kircali, F., 2004. Comparison of crude and excretory/secretory antigens for the diagnosis of Fasciola hepatica in sheep by western blotting. *Turk. J. Vet Animal Sci*., 28, 943-949.

Hailu, D., 1995. Bovine fascioliosis at Awassa municipal slaughter house prevalence and economic loss. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM thesis.

Hillyer, G. V., 1999. Immuno diagnosis of human and animal Fascilosis. In: Dalton, J. (Ed), Fasciolosis ,13. CAB international, Pp. 435-447.

Hillyer, G.V., Soler de Galanes, M., Buchon, P., Buorland, J., 1996. Herd evaluation by enzyme-linked immunosorbent assay for the determination of *Fasciola hepatica* infection in sheep and cattle from the Altiplano of Bolivia. *Vet. Parasitol*., 61(3-4), 211–220. https://doi.org/10.1016/0304-4017(95)00831-4.

Ibarra, F., Montenegro, N., Vera, Y., Boulard, C., Quiroz, H., Flores, J., Ochoa, P., 1998. Comparison of Three ELISA tests for Seroepidemiology of bovine fascioliosis. *Vet. Parasitol*., 77(4), 229-236. https://doi.org/10.1016/S0304-4017(98)00111-3.

Kerr, M.G., 2002. Clinical enzymology-plasma enzymes in diagnosis, pp: 135-296. In: M.G. Kerr, (ed.). Veterinary Laboratory Medicine (Clinical Biochemistry and Haematology). 2nd ed., Blackwell Science. London, United Kingdom.

Malone, J.B., Gommes, R., Hansen, J., Yilma, J.M., Slingenberg, J., Snijders, F., Nachet, O. F. and Ataman, E., 1998. A Geographic information system on the potential distribution and abundance of *Fasciola hepatica* and *F. gigantica* in East Africa based on Food and Agriculture Organization databases. *Vet. Parasitol.*, 78(2), 87-101. https://doi.org/10.1016/S0304-4017(98)00137-X.

María Soledad, M.G., 1992. Epizootiologia de la Fasciolosis bovina en Asturias: Identificación y expresión de un antigeno unitario Universidad de Oviedo, Facultad de Biologia, PhD Tesis. https://www.educacion.gob.es/t.

Martinez, A., Martinez-Cruz, M.S., Martinez, F.G., Gutierrez, P.N., and Hernandez, S., 1996. Detection of antibodies to *Fasciola hepatica* excretory-secretory antigens in experimentally infected goats by Enzyme Immunosorbent Assay. *Vet. Parasitol.,* 62(3-4), 247-252. https://doi.org/10.1016/0304-4017(95)00876-4.

Merck., 2006. Merck Veterinary manual, 9th Edition, Merck and Co. Inc, Whitehouse Station., NJ, USA.

Mesfin, A. Y., 1999. Study on the prevalence of bovine Fasciolosis and morphometric analysis of liver fluke population in North Gondar area, Ethiopia. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia. DVM Thesis.

O'Neill, S.M., Brady, M.T., Callaghan, J.J., Mulcahy, G., Joyce, P., Mills, K. H.G., and Dalton, J.P., 2000. *Fasciola hepatica* infection down regulates th1 responses in mice. *Parasite Immunol.*, 22(3),147–155. doi.org/10.1046/j.1365-3024.2000.00290.x.

Ogunrinade, A., and Ogunrinade B., 1980. Economic importance of Bovine Fasciolosis in Nigeria, *Trop. Anim. Health* Prod., 12 (3). 155-160.

Perez, J., Ortega, J., Bravo, A., Diez-Banos, P., Morrondo, P., Moreno, T., and Martinez-Moreno, A., 2005. Phenotype of hepatic infiltrates and hepatic lymph nodes of lamb primarily and challenge infected with *Fasciola hepatica*, with and without triclabendazole treatment. *Vet. Res*., 36(1), 1-12. doi.org/10.1051/vetres:2004047.

Rahmeto, A., 1992. Fasciolosis clinical occurrences, coprological, abattoir and snail survey in and around Wolisso. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia. DVM Thesis.

Ramnic Sood., 1999. Medical Laboratory Technology Methods and Interpretations 5th ed.. Jaypee Brothers Medical Pub.New Delhi, India. Pp 480-529.

Rickard, L.G., 1995. Development and application of a dot- ELISA test for the detection of serum antibodies to *Fasciola hepatica* antigens in llamas. *Vet Parasitol.,* 58(1-2), 9-15. doi.org/10.1016/0304-4017(94)00709-L.

Sanchez-Andrade Fernandez, R., Morrondo Pelayo, P., Lopez Sandez, C., Panadero Fontan R., Diez Banos P.,1995. Evaluation of *Fasciola hepatica* infection preva-

lence in cattle in Galicia (north west Spain) by enzyme linked immunosorbent assay. *Res. Rev. parasitol.*, 55 (2), 103-107.

Sanchez-Andrade, R., Paz-Silva A., Suarez, J.L., Pandero, R., Pedreira, J., Lopez, C., Diez-Banos, P., and Morrondo, P., 2002. Influence of age and breed on natural bovine Fasciolosis in an endemic area (Galicia, NW Spain). *Vet. Res. Commun.*, 26(5), 361-370.

Sisay, A., and Nibret, E., 2013. Prevalence and risk factors of bovine and ovine Fasciolosis, and evaluation of direct sedimentation sensitivity method at Bahir-Dar Municipal Abattoir, Northern Ethiopia. *Ethiop.Vet.J.,* 17(2), 1-17. dx.doi.org/10.4314/evj.v17i2.1.

Soulsby, E.J.L., 1982. Helminthes, arthropods and protozoa of domesticated animals, 7th Ed. Baillière Tindall, London, UK, Pp.40-52.

Thrusfield, M., 1995. Veterinary Epidemiology 2nd edition, University of Edinburgh,

Blackwell Science Ltd., UK. Pp.180-188.

Troncy, P.M., 1989. Helminths of livestock and poultry in Tropical Africa. In: Fischer. 1989. Manual of tropical veterinary parasitology. CAB International, UK. Pp. 63-73.

Urquhart, G.M., Duncan, J.L., Armour, J., Dunn., Jenning A.M., 1996. Veterinary parasitology. 2nd Edition. Blacwell Scince, Ltd., UK. Pp. 103-113.

Wondwosen, A., 1990. Prevalence of bovine Fasciolosis in Arsi administrative region. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM Thesis.

Yilma, J.M., and Malone, J.B., 1998. A geographic information system forecast model for strategic control of fasciolasis in Ethiopia. *Vet. Parasitol.*, 78 (2), 103-123. doi.org/10.1016/S0304-4017(98)00136-8

Zewdu, B., 1991. Prevalence and economic analysis of liver fluke infestation in cattle slaughtered at Jimma Municipal Abattoir. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia, DVM Thesis.

Prevalence, risk factors and antimicrobial susceptibility profile of *Salmonella* isolated from dogs of Ambo, Bako and Gojo towns of West Shoa, Ethiopia

Endrias Zewdu Gebremedhin[*1], Sisay Miheretu[2], Lencho Megersa[3], Edilu Jorga Sarba[1], Getachew Kebebew[1] and Solomon Shiferaw[3]*

[1]Department of Veterinary Sciences, College of College of Agriculture and Veterinary Science, Ambo University, P.O.Box 19, Ambo, Ethiopia.

[2]Department of Animal Production and Technology, College of Agricultural Sciences, Welkite University, P.O.Box 07, Welkite,Guragie Zone, SNNPR, Ethiopia.

[3]Department of Veterinary Laboratory Technology, College of Agriculture and Veterinary Science, Ambo University, P.O.Box 19, Ambo, Ethiopia

**Corresponding author: Endrias Zewdu Gebremedhin endrias.zewdu@gmail.com,*

Abstract

Salmonella is the most known zoonotic bacterial agent, which produces salmonellosis in animals as wells as in humans. The objectives of this study were to estimate the prevalence, to determine antimicrobial susceptibility and to assess risk factors associated with *Salmonella* shedding in dogs in selected towns of West Shoa Zone, Oromia, Ethiopia. Using a cross-sectional design, a total of 438 rectal swab samples were collected from randomly selected dogs for isolation and identification of *Salmonella* using standard procedures. A questionnaire survey was also administered. The results showed that 48 (11.0%, 95%, [CI]: 8.2% - 14.3 %) dogs were positive for *Salmonella*.The occurrenceof *Salmonella* was 10.9% (26/238), 11.6% (15/129) and 9.9% (7/71) in Ambo, Bako and Gojo towns respectively. *Salmonella* shedding was significantly higher in older dogs (15.0%) than younger ones (6.1 %) ($P<0.05$). The occurrence of *Salmonella* was not significantly associated with study towns, sex, breed of dogs,educational level of owners, presence of other domestic animals and housing system($P>0.05$). Multivariable logistic regression analysis showed that the odds of isolation of *Salmonella* was significantly higher in old than young dogs (OR): 2.6, 95% CI: 1.3-5.6, $P=0.007$) and in dogs eating mixed type of feeds (raw animal products, household leftover) than cooked feed (OR = 9.0, 95% CI: 1.2 – 68.1, $P=0.033$). There was also a significant difference between kebeles of the study areas in that *Salmonella* isolation was nearly nine times higher in Bako *Kebele* 02 ($P=0.005$) as compared to Ambo kebele 03. *Salmonella* isolates have developed the highest level of resistance for ampicillin (100%), tetracycline

(93.3%) and streptomycin (80.0 %), however, all isolates were susceptible to Norfloxacin. The occurrence of *Salmonella*, most of which resistant to commonly used antimicrobials, in rectal swab samples show the potential threat dogs may pose to public health in the study area.

Keywords: Antimicrobial Resistance; Dog; Ethiopia; Prevalence; Risk factor; *Salmonella*.

Introduction

Salmonella is the causative agent of both human and animal salmonellosis. The bacterium causes infections ranging from subclinical carrier status to acute fatal septicemia (Quinn *et al.*, 2011). It is a potential cause of acute and chronic diarrhea and death in numerous animal species and in human beings (McGavin *et al*., 2001). About 22 million diseases and 220,000 deaths were reported globally during 2000 as the result of typhoid fever (Crump *et al.,* 2004) and 93.8 million illness of gastroenteritis and 155,000 deaths due to non-typhoidal *Salmonella* (Kirk *et al.*, 2015). The main source of infection for *Salmonella* to human is feces of almost all animals including pets that have intimate relationships with humans (Ojo and Adetosoye, 2009).

The prevalence of subclinical carriage of *Salmonella* in apparently healthy dogs varies greatly among individual countries and has been reported to range from 0 to 44 % (Carter and Quinn, 2000). Most cases of *Salmonella* infections in dogs are latent and subclinical (Kozak *et al.*, 2003). More than one-third of healthy dogs have *Salmonella*in their digestive tract and shed the organisms (Finley *et al*., 2007). High numbers of the microorganism can reside in the intestines and mesenteric lymph nodes without clinical signs making an estimate of the prevalence of *Salmonella* in dogs in the community difficult to establish. Nevertheless, clinical cases of salmonellosis when seen is often associated with hospitalization, another infection or debilitating conditions in adults, or exposure manifested by watery or mucoid diarrhea with blood in severe cases, vomiting, fever, anorexia, lethargy, abdominal pain and progressive dehydration (Carter and Quinn, 2000).

In developing countries, control of antimicrobial-resistant infections in both humans and animals are of a great challenge because of inadequate adherence to prudent use of antimicrobials, unhygienic living conditions, close con-

tact and sharing of houses between animals and humans (Feasey *et al*., 2012). Different scholars reported antimicrobial resistant *Salmonella* isolates from categories of food (Weldo *et al*., 2015; Ejo *et al.,* 2016), animal (Abdi *et al*., 2017; Kiflu *et al.*, 2017) and human (Eguale *et al.,* 2015; Marami *et al*., 2018) specimens in Ethiopia. There is an increasing concern with this pathogen due to the emergence and spread of antimicrobial-resistant and potentially more pathogenic strains (WHO, 2012).

Salmonellosis influences the economy of both developing and developed countries through incurring a cost in relation to surveillance, prevention and treatment (Crump *et al.*, 2004) that requires epidemiological and microbiological investigation. Information about the isolation, risk factors for sheddingand antibiogram of *Salmonella* species in dogs is scarce in Ethiopia in general and totally absent in the current study areas (Ambo, Bako and Gojo towns). In this aspect, the investigation of *Salmonella* in dogs was thought to be important to see the contribution of dogs as a potential source of zoonotic *Salmonella* to humans. Therefore, the objectives of the present study were to estimate the prevalence of *Salmonella* in dogs, to assess the major risk factors and to determine antimicrobial susceptibility of the isolates.

Materials and methods

Study area

The study was conducted in three towns of West Shoa Zone of Oromia Region from December 2016 to April 2017 (Figure 1). These towns are namely Ambo, Gojo and Bako. Ambo is the administrative center of Ambo district and West ShoaZone located 114 Km West of Addis Ababa, the capital city of Ethiopia. The town had a total human population of 74, 843 out of which 39,192 are male and 35,651 are female. Ambo Zuria (Zuria; around) district has 110,952 cattle, 87,817 sheep, 48,937 goats, 3,419 equines and 40,971 chickenpopulations. Bako town, the administrative center of Bakotibe district is located 260 Km West of Addis Ababa.Based on figures from the Central Statistical Agency in 2005, Bako has an estimated total population of 18,641 of whom 9,370 are men and 9,271 women. Bakotibe district has 137,343 cattle, 12,502 sheep, 24,212 goats, 3,685 horses, 8,415 donkeys, 1,023 mule and 96,742 poultry (Bekele and Kebede, 2016). Likewise, Gojo town is the administrative center of Jeldu district of West Shoa Zone located 120 Km West of Addis Ababa.The human population of Jeldu district is 202,655 of which 102,796 are females and the remaining 99,859 males (CSA, 2008).Jeldudistrict has a total population of

78,300 cattle, 50,450 sheep, 25,560 goats, and 45,210 equine species.There was no accessible recorded statistical information on dog population in the study areas. The three towns have bimodal rainfall characterized by a small rainy season from February to May and a big rainy season from July to September. The dry season extends from October to January (CSA, 2007; CSA, 2015).

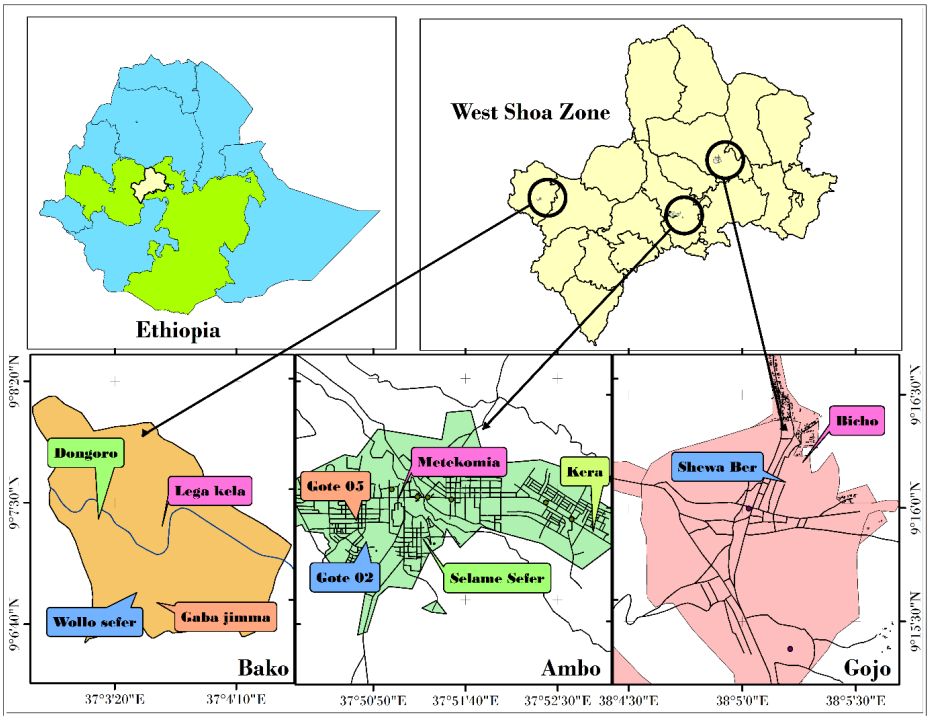


Figure 1. Map of the study towns indicating sampled Kebeles

Study population

The study population was all dogs found in the Ambo, Bako and Gojo towns. Apparently healthy dogs of differentage groups, breed and bothsexesfound in the selected Kebeles (the smallest formal administrative unit of a town) of the three towns were considered as the target population.

Study design

A cross-sectional study was conducted to assess the prevalence of *Salmonella* from rectal swab samples of dogs. The total sample size was divided into the three towns proportionally depending on the number of Kebeles. In each Kebeles, Gotes were approached (a Gote is a subset of Kebele comprising up to 50 households). Ambo, Bako and Gojo towns have three, two and one Kebeles respectively. From each Kebele, two Gotes were randomly selected.Twelve Gotes were selected with an almost equal number of samples in order to decrease bias.

Sample size determination

The sample size was determined using the formula described by Thrusfield (2007) with the assumption of 50% expected prevalence, 95% confidence interval and 5% absolute precision.

Where n= required sample size, p=expected prevalence d= desired absolute precision. Therefore, the calculated sample size was 384. About 15% of the calculated sample size was additionally taken to reduce the risk of sample loss during analysis. Thus, the total sample size in this study was 438.

Sample collection

Rectal swab sample was collected from each dog after proper restraining. Thirty samples were collected and processed per week and 438 samples were collected from different sources. The samples were collected in sterile buffered peptone water (HiMedia, India) using sterile swabs and transported to Ambo University Veterinary Laboratory in icebox containing ice packs.

Questionnaire survey

A pre-tested questionnaire was administered for dog owners in order to collect information about management risk factors such as feeding (cooked animal products and mixed [raw meat, cooked animal products and household left over]), housing (indoor, outdoor and mixed type), educational status of owners (Illiterate, Elementary School, Secondary School, and Tertiary/University), treatment status of dogs with antibiotics (treated and not treated), presence of other animals (present and absent) within the same household, breed and

age of dogs. Age was categorized as young (dogs of less than and equal to two years) and adult (more than two years) (Wikipedia, 2019).

Inclusion and exclusion criteria

Accessible dogs whose owners are willing for sampling were included in the study while critically sick dogs and those under treatment were excluded.

Isolation and identification of *Salmonella*

Isolation and identificationof *Salmonella* from rectal swab samples were performed according to the procedure recommended by the international standard organization (ISO) for isolation of *Salmonella* (ISO 6579, 2007). Briefly, rectal swab samples were transferred into a tube with 9 ml of buffered peptone water (HiMedia, India), shaken for approximately 2 minutes and incubated at 37±1°C for 18 ± 2 h. A portion of the culture (0.1 ml) was transferred to a tube containing 10 ml selective enrichment liquid media (Rappaport-Vassiliadis, HiMedia, India) and incubated at 42°C for 24±3 h. Similarly, 1 ml of the culture was transferred to a tube containing 10 ml of Tetrathionate broth (Conda S.A., Spain) and incubated at 37°C for 24±3 h. A loopful of inoculum from each of enrichment cultures was then inoculated on the surface of two different plates, viz. xylose lysine deoxycholate (XLD) agar (Sisco research lab, India) and brilliant green agar (BGA) (HiMedia, India) and then incubated at 37°C for 24±3 h. For confirmation, presumptive *Salmonella* colonies from both XLD agar and BGA were selected and streaked onto the surface of pre-dried nutrient agar (Oxoid, England) plates and incubated at 37°C for 24±3 h. Colonies from nutrient agar were inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar (HiMedia, India), lysine iron agar (Conda S.A., Spain), Simmon's citrate agar (HiMedia, India), urea broth (HiMedia, India), Sulphide Indole Motility (SIM) medium (Sisco research lab, India) and incubated for 24 or 48 h at 37°C.

Colonies producing an alkaline (red) slant with acid (yellow) butt with hydrogen sulphide production (blackening) on TSI, positive for lysine (purple color with gas production), positive for citrate utilization (blue color), negative for urea hydrolysis (no color change) and negative for tryptophan utilization (Indole test) (yellow-brown ring) were considered asSalmonella (ISO 6579, 2002).

Antimicrobial susceptibility testing

The pure *Salmonella* isolates confirmed by the biochemical testing procedures were tested for antimicrobial susceptibility. Antimicrobial susceptibility testing was done using the agar disk diffusion method as described by the Clinical Laboratory Standard Institute (CLSI, 2014).Fourto five well-isolated colonies from nutrient agar plates were transferred intotubes containing 5 ml of nutrient broth (Conda S.A., Spain). The broth culturewas incubated at 37ºC for 4-6 h until it achieved the 0.5 McFarland turbiditystandards. A sterile cotton swab was dipped into the suspension, rotated severaltimes, pressing firmly on the inside wall of the tube above the fluid level toremove excess inoculum and swabbed uniformly over the surface of MullerHinton agar plate (HiMedia, India). The plates were held at room temperature for 15 min to allow drying. The discs were placed with 20 mm gap between them and 15 mm from the edge of the plates to prevent overlapping of the inhibition zones. The plates were incubated at 37 ºC for 24 h. The diameter of the zones of inhibitions was recorded and classified as resistant, intermediate or susceptible according to the interpretive standards of the Clinical Laboratory Standards Institute (CLSI, 2014)

Eleven antimicrobial disks, i.e. ampicillin (10 µg), amoxicillin (30 µg), norfloxacin (30µg), chloramphenicol (30 µg), vancomycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), sulfamethazine (300 µg), lincomycin (2 µg)penicillin (10 µg) and doxycycline (10 µg), currently available and commonly used as chemotherapeutic and chemoprophylactic drugs for *Salmonella* infection in humans and animals were used. The zone of inhibition was measured and reported as susceptible (S) intermediate (I) or resistant (R) in reference to the zone size of the interpretive chart.The selection of the drugs was based on the standards drugs used forthe treatment of salmonellosis (CLSI, 2014) and the availability of these drugs during the research work.Standard strains of *E. coli* ATCC 29522 and ATCC 35218 kindly provided by Ethiopian Public Health Institute were used as a quality control organism throughout the study for antimicrobial susceptibility testing.

Data analysis

All data collected were entered and coded using Microsoft Excel Spreadsheet. Statistical analysis was made using STATA software version 11.0 (STATACORP, 2009). Descriptive statistics was used to summarize the data. Chi-square and logistic regression analyses were used to assess the association of risk factors with the prevalence of *Salmonella*. For all risk factors, the level

with the lowest prevalence was used as a reference category. Those variables with *P*-value ≤ 0.25 in the univariable logistic regression analysis were further analyzed using multivariable logistic regression after checking for multicollinearity. In all the cases *P*< 0.05 was set for significance.

Results

Prevalence

The overall prevalence, as well as occurrence of *Salmonella* at town level in the rectal swab samples of 438 apparently healthy dogs, enrolled in the study was shown in Table 1.

Table 1. Prevalence of *Salmonella* infection in rectal swab samples of dogs of Ambo, Bako and Gojo towns, West Shoa Zone, Ethiopia.

Study towns	No. of dogs examined	No. positive	% prevalence (95% CI)
Ambo Town	238	26	10.9 (7.3 – 15.6)
Bako Town	129	15	11.6 (6.7 – 18.5)
Gojo Town	71	7	9.9 (4.1 – 19.3)
Total	**438**	**48**	**11.0 (8.2 - 14.3)**

No.= number, CI=confidence interval

Risk factors

Univariable logistic regression analysis showed that the risk of *Salmonella* shedding was significantly higher in older dogs than in young dogs (OR = 2.7, *P*=0.004). Similarly, there was a significant difference in the prevalence of *Salmonella* shedding in dogs between Kebeles of the study areas (*P*<0.05). Feed type is borderline significant (*P*=0.053) i.e., the probability of shedding *Salmonella* is higher in dogs feeding on mixed type of feed (raw animal products and household leftover) than dogs fed on cooked animal products. However, there was no significant difference inthe prevalence of *Salmonella* with respect to towns, breed, sex, educational status of owners, presence of other animals in the household, and housing system of dogs (*P*>0.05) (Table 2).

Table 2. Results of univariable logistic regression analysis of potential risk factors for *Salmonella*sheddingof dogs in Ambo, Bako and Gojo towns

Variables	Category	No. pos./tested (%)	OR	95% CI	P-value
Town	Gojo	7/71 (9.9)	1	-	-
	Ambo	26/238 (10.9)	1.1	0.5- 2.7	0.799
	Bako	15/129 (11.6)	1.2	0.5-3.1	0.702
Kebele	Ambo 03	2/72 (2.8)	1	-	-
	Gojo 01	7/71 (9.9)	3.8	0.8-19.1	0.102
	Bako 02	9/86 (10.5)	4.1	0.9-19.6	0.083
	Bako 01	7/66 (10.6)	4.2	0.8-20.8	0.078
	Ambo 01	8/63 (12.7)	5.1	1.0-24.9	0.045
	Ambo 02	15/80 (18.8)	8.1	1.8-36.7	0.007
Age	Young	12/198 (6.1)	1	-	-
	Old	36/240 (15.0)	2.7	1.4-5.4	0.004
Breed	Cross	6/77 (7.8)	1	-	-
	Local	42/361 (11.6)	1.6	0.6-3.8	0.331
Sex	Female	8/103 (7.8)	1	-	-
	Male	40/335 (11.9)	1.6	0.7-3.6	0.239
Educational status of the owner	Illiterate	6 (10.5)	1	-	-
	Primary school	13 (12.12)	1.1	0.4-3.0	0.833
	Secondary school	14 (12.2)	1.3	0.6-2.8	0.522
	Tertiary/ university	15 (9.4)	1.3	0.6-2.9	0.502
Presence of other animals in the HH	No	21 (10.8)	1	-	-
	Yes	27 (11.1)	1.0	0.6-1.9	0.936
Housing system	Indoor	12/79(15.2)	1	-	-
	Outdoor	22/176(12.5)	1.7	0.9-3.5	0.130
	Mixed	14 (7.7)	2.2	1.0-5.0	0.066
Feed type	Cooked animal feed	1 (1.9)	1	-	-
	Mixed	47 (12.2)	7.2	1.0-53.5	0.053

OR= Odds ratio, No. pos.= number positive, CI= Confidence interval, HH=household, mixed= raw meat, cooked animal products, and household leftover

The variables town, breed, educational status of dog owners and presence of other animals in the household were excluded from entry into the multivari-

able logistic regression model due to univariable *P*-values >0.25. The variables Kebele, age, sex, housing system, and feed type were all non-collinear with univariable *P*-value <0.25 hence entered into the multivariable model. The final multivariable logistic regression model of risk factors analysis revealed that *Kebele*, age, and feed type had a significant association with *Salmonella* prevalence and hence are independent predictors (*P*<0.05) (Table 3).

Table 3. Results of the multivariable logistic regression model for the predictors of *Salmonella*s hedding in dogs in Ambo, Bako and Gojo towns

Variables	Category	aOR	95% CI	P-value
Kebele	Ambo,03	1.0	-	-
	Gojo,01	4.1	0.9-20.4	0.077
	Bako,02	9.0	1.9-41.9	0.005
	Bako,01	4.9	1.0- 24.4	0.055
	Ambo,01	3.9	0..7-19.6	0.105
	Ambo,02	3.7	0.7-18.9	0.113
Age	Young	1.0	-	-
	Old	2.6	1.3-5.3	0.007
Sex	Female	1.0	-	-
	Male	1.7	0.8-3.9	0.203
Housing	Indoor	1.0	-	-
	Outdoor	2.0	1.0-4.3	0.063
	Mixed	2.2	0.9-5.1	0.079
Feed type	Cooked feed	1.0	-	-
	Mixed	8.6	1.1-65.4	0.037

aOR = adjusted odds ratio, CI= confidence interval

Antimicrobial susceptibility tests

The result of antimicrobial susceptibility testing of Salmonella isolated from dogs using 11 selected antimicrobial drugs is presented in Table 4. Fifteen Salmonella isolates proportionally selected among the whole isolates of the three townswere tested for antimicrobial susceptibility testing. The highest level of resistance was observed for ampicillin (100%), penicillin (93.3%), streptomycin (80%) and lincomycin (66.7%). All isolates were susceptible to norfloxacin.

Table 4. Results of antimicrobial susceptibility testing of *Salmonella* isolates (n=15) from rectal swab samples of dogs in Ambo, Bako and Gojo towns

Antimicrobial class	Antimicrobial discs and concentration	Salmonella isolates (n=15)		
		No Resistant (%)	No Intermediate (%)	No susceptible (%)
Aminoglycosides	Streptomycin (10 µg)	12 (80)	(0)	3 (20)
ß-lactams	Amoxicillin (20 µg)	9 (60)	5 (33.3)	1 (6.7)
	Ampicillin (10 µg)	15 (100)	0 (0)	0 (0)
	Penicillin (10 µg)	14 (93.3)	0 (0)	1 (6.7)
Phenicols	Chloramphenicol (30 µg)	3 (20)	11 (73.3)	1 (6.7)
Tetracyclines	Tetracycline (30 µg)	13 (86.7)	-	2 (13.3)
	Doxycycline (10µg)	6 (40)	8 (53)	1 (6.7)
Folate pathway inhibitors	Salphametazone (25µg)	2 (13.3)	0 (0)	13 (86.7)
Fluoroquinolones	Norfloxacin (10 µg)	0 (0)	0 (0)	15 (100)
Lincosimide antibiotics	Lincomycin (2 µg)	10 (66.7)	4 (26.7)	1 (6.7)
Glycopeptides	Vancomycin (30 µg)	8 (53.3)	6 (40)	1 (6.7)

Six different multidrug resistance patterns were observed among *Salmonella* isolates tested. About 13.3% of *Salmonella* isolates tested showed multidrug resistance to six different classes of drugs. Two isolates were resistant to six antimicrobial classes (Table 5).

Table 5. Multiple drug resistance pattern of *Salmonella* isolates from rectal swab samples of dogs in Ambo, Bako and Gojo towns

Number	Antimicrobial resistance pattern (No.)	No. of isolates resistant (%)
Three	AMP,TET, S	1 (6.7)
Four	AMP,TET, PB, S	2 (13.3)
Four	AMP,TET, PB, VA	7 (46.7)
Five	AMP,TET, PB, VA, C	2 (13.3)
Six	AMP,TET, PB, VA, C, S	1 (6.7)
Six	AMP,TET, PB, VA, S, SXT	2 (13.3)

AMO: Amoxicillin, C: Chloramphenicol, PB: Lincomycin, NOR: Norfloxacin, P: Penicillin, VA: Vancomycin, SXT: Sulfamethazine, S: Streptomycin, AMP: Ampicillin, TET: Tetracycline, DOX: Doxycycline.

Discussion

Dogs have been reported to be the carrier of *Salmonella* worldwide which have the potential to serve as sources of exposure or infection for other animals (Carter and Quinn, 2000). It was reported that the intestinal carriage of *Salmonella* by dogs is more common than the prevalence of clinical disease (Kozak *et al.*, 2003). The frequency of rectal swab isolation of *Salmonella* from clinically healthy dogs was reported to be between 0.0% and 43.0% (Carter and Quinn, 2000; Sanchez *et al.*, 2002).

In Ethiopia, like many other developing countries, dogs are predominantly kept for guarding/security and hunting. Feeding, housing, and health care their owners provide are generally inadequate**,** consequently,most dogs have free access to feed and contaminate the environment through open-air defecation (Castro *et al.*, 2019). The percentage of *Salmonella* shedding in this study (11.0%) might be an underestimate due to the fact that culture-negative dogs might still be sub-clinical shedders since the bacteria are shed intermittently and sampling was done only once and swab rather than faeces was examined (Carter and Quinn 2000).

The present study indicated an overall *Salmonella* prevalence of 11.0% which is higher than the reports ofSeepersadsingh*et al.* (2004) in subclinical dogs (3.6%) in Trindad, Amadi *et al.*(2018)in the feces of non-diarrheic owned dogs in Grenada (5.6%) and from apparently healthy dogs in Midlands region of the United Kingdom (0.2%) (Lowden *et al.*, 2015). Kiflu *et al.* (2017) from Addis Ababa reported 11.7% *Salmonella* prevalence in apparently healthy dogs, which is in accord to the present study. However, Aliyi *et al.* (2018) reported higher prevalence from dogs of Holeta town.

This difference in prevalence of *Salmonella* in dogs among different studies in various countries mightbe attributed to variation in hygiene, sanitation procedures, or seasonof sampling (Lowden *et al.*, 2015), the number of dogs sampled, type of sample, geographical properties, sampling strategies and isolation methods employed (Seepersadsingh *et al.*, 2004).

Studies conducted on prevalence of *Salmonella* in Ethiopia reported prevalence of 7.8% in food items (Zewdu and Cornelius, 2009) and 17.7% from carcass swab (Weldo *et al.*, 2015) while prevalence of 7.4% from stool samples (Zewdu and Cornelius, 2009), 12% from abattoir workers and meat retailers (Ejo

et al., 2016) were reported. These reports indicate that *Salmonella* is prevalent in animals, humans and food items in different parts of Ethiopia that could support the present study in that *Salmonella* can be prevalent in dogs (Addis *et al*., 2011; Wabeto *et al.,* 2017).

Dogs shedding *Salmonella* might have acquired the organism from their feed or from materials ingested during scavenging. Scavengers exposed to many contaminated materials are likely to harbor more *Salmonella* serovars than non-scavengers kept under hygienic conditions. The hypothesis behind was that the prevalence of *Salmonella* would be higher in dogs eating a mixed diet than eating cooked animal products. In the present study, the prevalence of *Salmonella* was significantly higher in dogs on the mixed diet (raw animal products, cooked animal products, andhousehold leftover) 47/385(12.2%) as compared to dogs on cooked animal product diet 1/53(1.9%) (Table 2).*Salmonella* has been isolated from 80.0% of bone and raw food diets and from 30% of the fecal samples from dogs fed the diet (Joffe and Schlesinger, 2002). Therefore, it implies that dogs fed raw animal products might be a source of environmental contamination with *Salmonella*.

In this study, age of dogs was another risk factor for *Salmonella* shedding, in that the prevalence was significantly higher in older (15.0%) than younger dogs (7.8%) (P<0.05). This could probably be associated with the longer time of exposure of older dogs to different risk factors of *Salmonella* and insufficiency of the immune response in older dogs to completely eliminate the bacteria from their body,in which case they carry the bacteria sub-clinically (Radostits*et al.,* 2006).Unlike this study, Castro *et al.* (2019) from Mexico recently reported that dog under one year aremore likely to acquire *Salmonella* than dogs older than one year. On the other hand, Aliyi *et al*. (2018) did not find a significant difference in the prevalence of *Salmonella* between age groups examined.

There was also a significant difference in the rate of isolation of *Salmonella* between *Kebeles*, which might be due to more availability of contaminated food leftover from customers in hotels, restaurants, and other cafes in those Kebeles where the isolation rate was higher. The difference might also be due to the high number of butchers and meat retailers, which might lead to more contamination of dog feeds with *Salmonella* in those *Kebele* where the isolation rate was higher.

In the present study, a significant difference in the shedding of *Salmonella* was not found with respect to the housing system of dogs. However, studies frequently report that faecal shedding is less frequent in household pet dogs as compared to those inkennels and stray dogs (Carter and Quinn 2000).

The development of multiple antimicrobial resistance seen in this study signals an important health threat in both the veterinary and public health sectors as it limits antimicrobial drugs available for effective control of Salmonella infections. Thus, regular monitoring of antimicrobial resistance is important because of the public health impact (Magiorakos et al., 2012) and the influence of such testing on the choice of antimicrobial drugs to treat clinical cases (Seepersadsingh et al., 2004).

The *Salmonella* isolates in this study had marked antimicrobial resistance to sixof the eleven antimicrobials tested (i.e. ampicillin, tetracycline, streptomycin, lincomycin, penicillin, and amoxicillin) and two isolates had multidrug resistance to six antimicrobials. Such high level of antimicrobial resistancein towns where dog owners do not visit veterinary clinics for health issues of their dogsis alarming as these dogs could potentially facilitate spreading of antimicrobial-resistant *Salmonella* to humans and other animals. A previous study from apparently healthy dogs visiting veterinary clinics in Addis Ababa for anti-rabies vaccination reported 30.9%, 38.1%, 59.5% resistance for ampicillin, streptomycin, and oxytetracyclines (Kiflu *et al*., 2017) which is lower than the present study.Since dogs can acquire *Salmonella* from their feed, especially raw animal products, they could be infected or receive drug-resistant *Salmonella* from the food chain or from the environment they are living because of improper use of antimicrobials and residues in animal products. In addition, the drug resistance of *Salmonella* from dogs can vary widely depending on the geographic area, level of sanitation, public awareness, socioeconomic status of owners and possibly, serovars (Kiflu *et al*., 2017). In the present study, norfloxacin had the best antimicrobial activity against *Salmonella* isolates. All 15 (100%) isolates were susceptible to norfloxacin. This result was comparable to the previous report on isolates of *Salmonella* from dogs (Seepersadsingh *et al.,* 2004).

The detection of *Salmonella* from rectal swab samples of Ethiopian dogs is of great significance towards the understanding of the possible role of dogs in the environmental spread and transmission to other animals and people who have close contact with carrier dogs. Such transmission is more likely to occur in

children and immunosuppressed individuals who are at higher risk of infection (Sato *et al*., 2000).

The current study did not identify *Salmonella* isolates to serotype level due to lack of laboratory facility. Not all antimicrobial drugs recommended for *Salmonella* were included in the antimicrobial susceptibility testing due to limited availability. Nevertheless, despite these limitations, the results on prevalence, risk factors, and antibiogram are still important since there is limited information in Ethiopia.

Conclusions

The prevalence of *Salmonella* in rectal swab samples of apparently healthy dogs of the study towns was moderately high. Age of dogs, Kebeles where dogs live and type of dog feeds are predictors of *Salmonella* isolation.The high rate of antimicrobial-resistant *Salmonella* could be a potential threat for humans and animals in the study areas.Establishment and enforcement of legislation, which encourages responsible dog ownership, education of dog owners, and further detailed studies on serotyping, molecular epidemiology of *Salmonella* isolates and surveillance of antimicrobial resistance are suggested.

Ethics approval and consent to participate

The ethical committee for animal experimentation of the Ambo University, Ethiopia (Ref. No. RD/AREC/004/2016) approved this research project. All animals were handled strictly in accordance with the good animal handling practice in order to minimize animal sufferings during sampling. Informed verbal consent was obtained from the dog owners.

Acknowledgments

This study was supported by Ambo University, Research and community service vice president office. We greatly thank dog owners and workers of animal health centers in Ambo, Bako and Gojo towns for their cooperation during sample collection.

Conflict of interest

There is no conflict of interest to declare

References

Abdi, R. D., Mengstie, F., Beyi, A. F., Beyene, T., Waktole, H., Mammo, B. and Abunna, F., 2017. Determination of the sources and antimicrobial resistance patterns of *Salmonella* isolated from the poultry industry in Southern Ethiopia. *BMC Infect. Dis*., 17, 352-352.

Addis, Z., Kebede, N., Worku, Z., Gezahegn, H., Yirsaw, A. and Kassa, T., 2011. Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross-sectional study. *BMC Infect. Dis*., 11:222.

Aliyi, S., Hirpa, E. Zewude, O. and Equar, Y. (2018).Isolation and Identification of *Salmonella* from Apparently Healthy Pet Dogs in Holeta Town, Western Shoa, Ethiopia.*Curr Trends Biomed. Eng and Biosci* 15(2): 555907. DOI: 10.19080/CTBEB.2018.15.555907.

Amadi, V. A., Hariharan, H., Arya, G., Matthew-Belmar, V., Nicholas-Thomas, R., Pinckney, R., Sharma, R. and Johnson, R. 2018. Serovars and antimicrobial resistance of non-typhoidal Salmonella isolated from non-diarrhoeic dogs in Grenada, West Indies. *Vet. Med. Sci.,* 4, 26-34.

Bekele, D. and Kebede, K., 2016. On-Farm Phenotypic Characterization of Indigenous Cattle in Bako Tibe and Gobu Sayo Districts of Oromia Region, Ethiopia. *J.Biol., Agri. and Healthcare*. 6 (19), 94-103.

Carter, M. E. and Quinn, P. J. 2000. *Salmonella* infections in dogs and cats, *Salmonella* in domestic animals, 231-244.

Castro, N. K.M., Muñoz, E.T., García, G.F., Herrera Ramírez, J.C., Valencia, G.L., Medina Basulto, G.E., Pujol Manríquez, L.C., Rentería Evangelista, T.B. (2019). Prevalence, risk factors, and identification of *Salmonella* spp. in stray dogs of northwest Mexico. *Austral. J. Vet. Sci.,* 51, 37-40.

Chen, B. Y., Wang, C. Y., Wang, C. L., Fan, Y. C., Weng, I. T. and Chou, C. H., 2016. Prevalence and Persistence of *Listeria monocytogenes* in Ready-to-Eat Tilapia Sashimi Processing Plants. *J. Food. Prot.,* 79, 1898-1903.

CLSI, 2014. M100-S24 Performance Standards for Antimicrobial Susceptibility Testing: Twenty Fourth Information Supplement, 68-150.

Crump, J. A., Luby, S. P. and Mintz, E. D., 2004. The global burden of typhoid fever. *Bull. World Health Organ*., 82, 346-353.

CSA (Central Statistics Agency of Ethiopia), 2007. Population and Housing Census of Ethiopia. http://www.csa.gov.et/newcsaweb/images/documents/surveys/Population%20and%

CSA, 2015. Cities & Towns [Online]. Available: https://www.citypopulation.de/Ethiopia.html [Accessed October 19, 2018].

CSA, 2008. Summary and Statistical Report of the 2007 Population and Housing Census Results. Addis Ababa, Ethiopia

Eguale, T., Gebreyes, W. A., Asrat, D., Alemayehu, H., Gunn, J. S. and Engidawork, E., 2015. Non-typhoidal *Salmonella* serotypes, antimicrobial resistance, and coinfection with parasites among patients with diarrhea and other gastrointestinal complaints in Addis Ababa, Ethiopia. *BMC Infect. Dis*., 15, 497.

Ejo, M., Garedew, L., Alebachew, Z. and Worku, W., 2016. Prevalence and antimicrobial resistance of *Salmonella* isolated from animal-origin food items in Gondar, Ethiopia. *Biomed. Res. Int*., 2016.

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S. and Gordon, M. A., 2012. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet*, 379, 2489-2499.

Ferede, B., Desisa, F., Feleke, A., Tadesse, G. and Moje, N. 2015. Prevalence and antimicrobial susceptibility of *Salmonella* isolated from apparently healthy slaughtered goats at Dire Dawa municipal abattoir, Eastern Ethiopia. *J. Microbiol. Antimicrob.,* 7, 1-5.

Finley, R., Ribble, C., Aramini, J., Vandermeer, M., Popa, M., Litman, M.and Reid-Smith, R. 2007. The risk of salmonellae shedding by dogs fed Salmonella-contaminated commercial raw food diets. *Can. Vet. J.*, 48, 69-75.

International Organization for Standardization (ISO) 6579, 2002. Microbiology of Food and Animal Feeding Stuff-Horizontal Method for the Detection of *Salmonella*, International Organization for Standardization (ISO), Geneva, Switzerland, 4th edition, Pp. 897-902.

ISO, 2007. ISO 6579 Microbiology of food and animal feeding stuff - Horizontal method for detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage, 14.

Joffe, D. J., and Schlesinger, D. P., 2002. Preliminary assessment of the risk of Salmonella infection in dogs fed raw chicken diets. *Can. Vet. J.*, 43, 441-442.

Kiflu, B., Alemayehu, H., Abdurahman, M., Negash, Y. and Eguale, T., 2017. *Salmonella* serotypes and their antimicrobial susceptibility in apparently healthy dogs in Addis Ababa, Ethiopia. *BMC Vet. Res.,* 13, 134.

Kirk, M. D., Pires, S. M., Black, R. E., Caipo, M., Crump, J. A., Devleesschauwer, B., Döpfer, D., Fazil, A., Fischer-Walker, C.L., Hald, T., Hall, A.J., Keddy, K.H., Lake, R.J., Lanata, C.F., Torgerson, P.R., Havelaar, A.H. and Angulo, F.J. 2015. Correction: World Health Organization Estimates of the Global and Regional Disease

Burden of 22 Foodborne Bacterial, Protozoal, and Viral Diseases, 2010: A Data Synthesis. *PLoS Med.*, 12, e1001940.

Kozak, M., Horosova, K., Lasanda, V., Bilek, J. and Kyselova, J., 2003. Do dogs and cats present a risk of transmission of salmonellosis to humans? *Bratisl. Lek. Listy.*, 104(10), 323-328.

Lowden, P., Wallis, C., Gee, N. and Hilton, A., 2015. Investigating the prevalence of Salmonella in dogs within the Midlands region of the United Kingdom. *BMC Vet. Res.*, 11, 239.

Magiorakos, A.-P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J.,Struelens, M.J.,Vatopoulos, A., Weber, and Monnet, D.L. (2012).Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance.*Clin Microbiol Infect*., 18, 268–281.

Marami, D., Hailu, K. and Tolera, M., 2018. Prevalence and antimicrobial susceptibility pattern of Salmonella and Shigella species among asymptomatic food handlers working in Haramaya University cafeterias, Eastern Ethiopia. *BMC. Res. Notes,* 11, 74-74.

Mcgavin, D. M., Carlton, W. W. J. and Zachary, J. F. 2001. Thompson's Special Veterinary Pathology, Mosby, Philadephia, USA.

Ojo, O. E., and Adetosoye, A. I., 2009. *Salmonella* Typhimurium infection in diarrhoeic and none-diarrhoeic infection dogs in Ibadan, Nigeria dogs in Ibadan, Nigeria. *Veterinarski Arhiv*., 79, 371-377.

Quinn, P., Markey, B., Leonard, F., Fitzpatrick, E., Fanning, S. and Hartigan, P., 2011. Veterinary Microbiology and Microbial Disease, 2nd Edition, Hometra, Syndey.

Radostits, O. M., Gay, C.C., Hinchcliff, K. W. and Constable, P. D. 2006. Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th editions, Saunders Ltd., USA.

Sanchez, S., Hofacre, C. L., Lee, M. D. and Maurer, J. J., 2002. Animal sources of salmonellosis in humans. *JAVMA*, 221, 492-497.

Sato, Y., Mori, T., Koyama, T. and Nagase, H. (2000). *Salmonella Virchow* infection in an infant transmitted by household dogs. *J. Vet. Med Sci,*. 62 (7), 767–769.

Seepersadsingh, N., Adesiyun, A. A. and Seebaransingh, R., 2004. Prevalence and antimicrobial resistance of Salmonella spp. in non-diarrhoeic dogs in Trinidad. *J. Vet. Med. B. Infect. Dis. Vet. Public Health*, 51, 337-342.

Statacorp, T., 2009. Statistical Software: Release 11 College Station, College Station, TX: StataCorp LP., USA.

Thrusfield, M., 2007. Veterinary Epidemiology; Describing disease occurrence, 3rd editions, Blackwell Science Ltd, Oxford, UK.

Wabeto, W., Abraham, Y. and Alemayehu, A. A., 2017. Detection and identification of antimicrobial-resistant Salmonella in raw beef at Wolaita Sodo municipal abattoir, Southern Ethiopia. *J. Health Popul. Nutr.*, 36-52.

WHO, (2012). The evolving threat of antimicrobial resistance: options for action. World Health Organization. https://apps.who.int/iris/handle/10665/44812.

Wikipedia. 2019. Aging in dogs. Retrieved from https://en.wikipedia.org/wiki/Aging_in_dogs, Accessed June 10, 2018.

Zewdu, E. and Cornelius, P., 2008. Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. *Trop. Anim. Health Prod.*, 41, 241-249

Prevalence and Risk Factors of Ectoparasites in Small Ruminants in and around Haramaya University, Eastern Oromia Region, Ethiopia

Adugna Abera[1] and Tsegay Tkue Gebrewahd[1]*

[1]College of Veterinary Medicine, Mekelle University, P.O. Box: 2080, Mekelle, Ethiopia

**Corresponding author: Tsegay Tkue Gebrewahd, Email: tsegay1984t@gmail.com*

Abstract

Infestation by external parasites causes mortality, decreased productivity and financial loss in the animal exports. A cross sectional study was conducted from November, 2016 to April, 2017 in and around Haramaya University, Eastern Oromia region, Ethiopia to determine the prevalence, host risk factors and identify ectoparasites on small ruminants. Accordingly, a total of 384 small ruminants (190 sheep, and 194 goats) were randomly selected and examined for the presence of ectoparasites. Then, samples of ectoparasites were collected manually and put in clean universal bottles containing 70% ethanol. The samples were transported to the Haramaya University Veterinary Parasitology laboratory and identified to genus/species level under stereomicroscope. The overall prevalence of ectoparasites was 98% (sheep 55.8% and goats 42.2%), and the major identified ectoparasites were ticks 80 (20.8%), lice 78 (20.3%) and fleas 70 (7.8%). The genus/species of the identified ectoparasites were lice (*Damalina ovis,* 12.8% and *Linognathus stenopsis,* 7.5%), fleas (*Ctenocephalus,* 7.8*), ticks (Ambyloma variegatum,* 6.8%, *Boophilus decoloratus,* 9.4%, *Rhipicephalus evertsi evertsi,* 2.9% and *Hyalomma truncatum,* 1.8%). Host risk factors such as sex, species, breed, body condition score and physiological status (pregnancy) were significantly associated ($p<0.05$) with the overall prevalence of ectoparasites in small ruminants. According to this study, there was high prevalence of ectoparasites in small ruminants of the study area. Therefore, to minimize this high prevalence of ectoparasites in small ruminants and their impacts, appropriate and strategic control measures are paramount important.

Keywords: Ectoparasites; Haramaya University; Prevalence; Risk factors; Small Ruminants

Introduction

Agriculture is the mainstay of Ethiopian economy employing over 80% of the adult population and accounts for 45% of the gross domestic production and 85% of the export earnings (Sertse, 2004). Sheep and goats are sources of foreign currency owing to skin export to the international markets. Moreover, Wool and manure are also important by-products of small ruminant productions (MoARD, 2005).

Small ruminant husbandry is constrained by compound effect of disease, poor feeding, and poor management leading to low rural income and low export income (Chalachew, 2001). Ectoparasites commonly ticks, mites, lice and ked are important parasites because of their disease transmission, blood feeding habit and skin damage in most of the livestock population (CSA, 2004). Ectoparasites of small ruminants cause blood loss and very heavy infestations result with severe anaemia. Moreover, they are the most important vectors of protozoan, bacterial, viral and rickettsial diseases (Radostits *et al*., 2007). All ectoparasites cause intense irritation to the skin, the extent depending on the parasite involved. Infested animals scratch, rub and bite the affected areas and this end up with skin damage (Radostits *et al*., 2007).

The utilization of skins in Ethiopia is estimated to be 75% and 97%, from goats and sheep respectively (Mohammed, 2000). However in recent years, it has been relegated because of skin rejection and down grading inflicted on hides and skin defects mainly due to infestation by external parasites causing mortality and decreased productivity (Kassa, 2006) and accounts for 75% financial loss in the animal exports. Around 1 million birr annual loss was estimated through rejection and down-grading of hides and skins in Ethiopia (Zeleke and Bekele, 2004). Keeping in view, little information on prevalence and importance of external parasites in small ruminant is available in and around Haramaya University, Oromia region. Therefore, the objectives of this study were: to estimate the prevalence of ectoparasites in small ruminants in the study area; to identify the ectoparasites to their genus/species level, and to assess the host risk factors for the prevalence of ectoparasites in small ruminants.

Materials and Methods

Study Area and Animals

The study was conducted in and around Haramaya University, Eastern Hararghe zone of Oromia Regional State of Ethiopia. According to the Zone Office of Agriculture and Rural Development, Haramaya is located approximately 527km East of Addis Ababa, and 14km West of Harare town. The elevation of the area is about 2000m above sea level, and geographically it is located at 41°59'58" latitude and 09°4'10" longitude. The livestock population of the area is Cattle: 63, 723 (40.8%) Sheep: 13, 612 (8.7%), Goats: 20, 350 (13%), Donkeys: 15, 975 (10.2%), Camels: 530 (0.3%), and Chickens: 42, 035 (27%). The study animals were sheep and goats population which were owned by Haramaya University and smallholder farmers under traditional management system (BoARD, 2017, unpublished data)

Study Design and Sampling

A cross sectional study was employed to address the objectives of the study. The study animals were selected by systematic random sampling technique and the required sample size was determined based on the expected prevalence of 50% and the formula given by Thrusfield (1995). The study considered 95% confidence interval and 5% precision level. Accordingly 384 sheep and goats were selected and for this study. The selected host risk factors were species, sex, age, breed, body condition score and physiological status (pregnancy).

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

Where

N=required sample size

P_{exp}=expected prevalence

d^2=desired absolute precision

Sample collection and identification

A total of 384 small ruminants (190 sheep and 194 goats) were randomly selected and studied for the presence of ectoparasites. Before clinical examination the age and body condition score of each animal was recorded. The age and body condition score (BSC) of each animal were determined based on the description given by Gatenby (2002) and Steele (1996), respectively. Then, clini-

cal examination was conducted by visual inspection and palpation of the skin for the presence of ectoparasites on all parts of the animal. Next, ectoparasites such as ticks, lice and fleas were collected by hand from their attachment sites and put in clean universal bottles with 70% ethanol (Urquhart *et al*., 1996). The collected samples were transported the Veterinary Parasitology Laboratory of Haramaya University, and examined under stereomicroscope for identification following the identification key given by Urquhart *et al*. (1996) and Walker *et al*. (2003).

Data management and analysis

Data obtained in the study was entered in to a computer on Microsoft Excel spread sheet. The frequencies of ectoparasites were compared with variables and expressed in percentage and subjected to p-value and chi-square (X^2) test using SPSS version 20. A p-value <0.05 was considered statistically significant association.

Results

Overall prevalence of ectoparasites in small ruminants

The overall prevalence of ectoparasites in small ruminants was 48.9%. The identified parasites were lice 78 (20.3%), ticks 80 (20.8%) and fleas 70 (7.8%). Four genera of ticks were identified (*Amblyomma variegatum, Boophilus decoloratus, Hyalomma truncatum and Rhipicephalus evertsi evertsi*), two genera of lice (*Linognathus stenopsis* and *Damalina ovis*) and one genus of fleas (*Ctenocephalus*) (Table 1). The hypothesized host risk factors (species, sex, breed, body condition score and pregnancy except age) have shown a statistically significant association with the overall prevalence of ectoparasites in small ruminants (Table 2).

Table 1. Overall and genus/species level prevalence of ectoparasites (N=384)

Genus	Species	Number of infested animals	Percent (%)
Damalina	Damalina ovis	49	12.8
Linognathus	Linognathus stenopsis	29	7.5
Ctenocephalus		70	7.8
Ambyloma	Ambyloma variegatum	26	6.8
Boophilus	Boophilus decoloratus	36	9.4
Rhipicephalus	Rhipicephalus evertsi evertsi	11	2.9
Hyalomma	Hyalomma truncatum	7	1.8

Table 2. Association of overall prevalence of ectoparasites with hypothesized host risk factors

Risk factors	Category	Number of examined	Number of positive (%)	X^2	95 % CI	p-value
Species	Ovine	190	55.8	7.023	0.008, 0.012	0.011
	caprine	194	42.3			
Sex	Male	202	55.4	7.178	0.007,0.010	0.008
	Female	182	41.8			
Breed						
Goats	BC	92	48.9	18.751	0.000,0.001	0.001
	AC	16	68.8			
	HHG	86	30.2			
Sheep	BOH	144	54.2			
	HHS	46	60.9			
Age	Young	121	53.7	1.602	0.223,0.240	0.206
	Adult	263	46.8			
Body condition	Poor	69	78.3	58.602	0.000,0.000	0.000
	Moderate	244	50.8			
	Good	71	14.1			
Pregnancy status	Pregnant	132	37.1	11.278	0.000,0.001	0.001
	Non-pregnant	252	55.2			

ey: BHO: Black Head Ogaden, HHS: Hararghe Highland Sheep, BC= Boer Cross, AC: Anglo Cross, HHG: Hararghe Highland Goat

Prevalence of ectoparasites by species and sex of small ruminants

According to the results of the study, there was statistically significant difference ($p<0.05$) in the prevalence of lice between species of small ruminants, where it was more prevalent in ovine more than caprine. Moreover, statistically significant difference ($p<0.05$) was found in the prevalence of ticks and lice between sex of small ruminants, where it was higher is females than males (Table 3).

Table 3. Prevalence of ectoparasites by species and sex of small ruminants

Ectoparasites	Species		X2 (P-value)	Sex		X2 (P-value)
	Ovine (n=106)	Caprine (n=82)		Male (n=112)	Female (n=76)	
Ticks	23.7%	18.0%	1.85(0.173)	24%	55%	24.95(0.000)
Lice	25.8%	14.9%	8.13(0.017)	38.5%	44.4%	1.76(0.415)
Fleas	6.3%	9.3%	1.17(0.280)	21.8%	10.2%	3.03(0.082)

n: Number of animals examined

Prevalence of ectoparasites by breeds of small ruminants

The overall prevalence of ticks and lice infestations in the different breeds of small ruminants was highly significant. Ticks infestation was more prevalent in Hararghe highland sheep, Boer cross and Anglo cross breeds of goats. Lice were more prevalent in black head Ogaden sheep and Hararghe highland goats (Table 4).

Table 4. Prevalence of ectoparasites by breeds of small ruminants

Ectoparasites	Sheep breeds		X^2 (p–value)	Goat breeds			X^2 (p–value)
	BHO	HHS		BC	AC	HHG	
Ticks	14.6%	47.2%	22.430 (0.000)	50.0%	53.3%	11.2%	30.2(0.000)
Lice	34.3%	3.8%	20.656 (0.000)	0.0%	0.0%	7.0%	7.0(0.030)
Fleas	5.1%	9.4%	1.208 (0.000)	11.1%	20.0%	2.4%	2.4(0.302)

BHO: Black Head Ogaden, HHS: Hararghe Highland Sheep, BC= Boer Cross, AC: Anglo Cross, HHG: Hararghe Highland Goat

Prevalence of ectoparasites by age groups of small ruminants

It was revealed that the difference in the prevalence of lice and fleas between young and adult goats was statistically significant ($p<0.05$). Furthermore, ticks prevalence was higher ($p<0.05$) in adult than young sheep. However, fleas prevalence was higher ($p<0.05$) in young than adult sheep (Table 5).

Table 5. Prevalence of ectoparasites by age of small ruminants

Ectoparasites	Goats		X2 (p-value)	Sheep		X2 (p-value)
	Young	Adult		Young	Adult	
Ticks	13.2%	20.6%	1.64 (0.945)	11.8%	30.3%	8.32 (0.004)
Lice	14.7%	15.1%	0.01(0.000)	29.4%	23.8%	1.23 (0.54)
Fleas	22.1%	1.4%	20.32(0.000)	14.7%	1.6%	12.60(0.000)

Prevalence of ectoparasites by body condition of small ruminants

This study has shown that the prevalence of lice and fleas was higher in small ruminants with poor than moderate and good body conditions ($p<0.05$). However, ticks prevalence was higher in small ruminants with moderate than poor and good body conditions (Table 6).

Table 6. Prevalence of ectoparasites by body condition of small ruminants

Ectoparasites	Sheep			X2 (p-value)	Goats			X2(p-value)
	P	M	G		P	M	G	
Ticks	12.2%	28.6%	12.5%	5.86 (0.053)	12.2%	28.6%	12.5%	5.86(0.053)
Lice	43.9%	22.6%	6.2%	15.12(0.004)	43.9%	22.6%	6.2%	15.12(0.004)
Fleas	14.6%	4.5%	0.0%	6.61(0.037)	14.6%	4.5%	0.0%	6.61(0.037)

P: Poor, M: Moderate, G: Good

Prevalence of ectoparasites by pregnancy status of small ruminants

It was also revealed that ticks prevalence was higher in pregnant than non-pregnant sheep ($p<0.05$). Likewise, lice prevalence was higher in pregnant than non-pregnant goats ($p<0.05$) (Table 7).

Table 7. Prevalence of ectoparasites by pregnancy status of small ruminants

Ectoparasites	Sheep		X2(p-value)	Goats		X2(p-value)
	Pregnant	Non-pregnant		Pregnant	Non-pregnant	
Ticks	40%	35.7%	24.15 (0.000)	17.9%	18.5%	0.01(0.994)
Lice	36%	19%	5.08 (0.280)	33.3%	13.0%	13.43(0.001)
Fleas	0.0%	4.8%	3.76 (0.152)	0.0%	11.1%	5.02(0.081)

Discussion

The overall prevalence of ectoparasites in both host species was 48.9% (sheep 55.8%, goats 42.3%). The animals were infested by single or mixed ectoparasites. The higher prevalence of ectoparasites in the study area could be due to the fact that sheep and goats could have frequent exposure to the same communal grazing land that favored the frequent contact and management system of animals. This finding is lower than the report of Fentahun *et al.* (2012), who reported an overall ectoparasites prevalence of 78.38% in small ruminants in and around Gondar Town, Ethiopia.

The prevalence of ectoparasites in sheep and goats in this study is lower than previous report by Bekele *et al.* (2011), who reported ectoparasites prevalence of 99.38% and 96.92% in sheep and goats, respectively in Wolmera district of Oromia region, central Ethiopia. However, the prevalence of ectoparasites in sheep in the current study is in close agreement with the report of Abebe *et al.* (2011), who reported 51.7% in Tigray region, Ethiopia. Moreover, Sertse (2004), reported ectoparasites prevalence of 50.5% in sheep in Kombolcha and Dessie of Amhara region, Ethiopia, which is comparable to the findings of the current study. The author also reported ectoparasites prevalence of 56.4% in goats, and this is higher than the prevalence of ectoparasites in goats (42.3%) in the present study. Similarly, Mulugeta *et al.* (2010) reported ectoparasites prevalence of 55.5% and 58% in sheep and goats, respectively, in Tigray region. According to this study, the identified ectoparasites were ticks 80 (20.8%), lice 78 (20.3%), and fleas 70 (7.8%). The prevalence of ticks in this study is in agreement with the report of Fentahun *et al.* (2012), which was found to be 20%. However, the authors reported lice and fleas prevalence of 54.6% and 35.7%,

respectively, which is not in line with the present findings. However, the prevalence of ticks in the present study is lower than the report of Abebe *et al*. (2011), who reported 48%. On the other hand, the prevalence of lice and fleas which was reported by the authors was 1.3% and 3.1%, respectively, which is lower than the findings of the present study.

In this study, four genera of ticks (*Ambyloma, Boophilus, Rhipicephalus and Hyalomma*) were identified which made a total prevalence of 23.7% and 18% in sheep and goats, respectively. This result is supported by previous reports of Teshome (2002), who reported ticks prevalence of 23.8% and 16% in sheep and goats, respectively in Sidama zone, southern Ethiopia. Additionally, Abebe *et al*. (2011), reported ticks prevalence of 40% and 58.8% in sheep and goats, respectively in selected districts of Tigray region, Ethiopia.

According to this study, it was revealed that the host risk factors such a species, sex, breed, body condition score, and physiological status (pregnancy) were found to have a statistically significant association with the overall prevalence of ectoparasites in small ruminants. However, Fentahun *et al*. (2012), reported species, sex and body condition score did not have statistically significant association with the overall prevalence of ectoparasites in small ruminants. But, they reported tick infestation was higher in adult than young small ruminants which contradict with the finding of the present study.

This difference might be due to factors such as difference in agro ecological zones and management systems of small ruminants. Moreover, Abebe *et al*. (2011), reported body condition score was statistically significantly associated with the overall prevalence of ectoparasites in small ruminants, where animals with poor body condition score were highly infested with single or mixed ectoparasites. This is also in line with the findings of the present study, where animals with poor body condition score were highly infested with ectoparasites than these with moderate and good body condition scores. This might be due to lowered immune response as a predisposing factor. The poor body condition could also be due to chronic ectoparasites infestation as the ectoparasites are blood suckers and cause skin irritation which in turn results animals to spend most of their time on itching (James *et al.,* 2002)

In the current study, host species was not found to have statistically significant association with the prevalence of ticks and fleas in small ruminants.

However, it was significantly associated with lice prevalence. However, age has shown highly significant association with the prevalence of ectoparasites in small ruminants. Likewise, Hailu *et al*. (2008) have reported higher fleas' infestation in young than adult small ruminants in and around Welaita Sodo, Southern Ethiopia. The higher prevalence of ectoparasites in the young small ruminants could be attributed due to the shorter hair and thin skin in which the fleas can find easy access to the skin and penetrate it without difficulty (Kusiluka and Kambarage, 1996).

Conclusion

This study was conducted to assess the prevalence of ectoparasites in small ruminants in and around Haramaya University, Oromia region, Ethiopia. The overall prevalence of ectoparasites in small ruminants was 48.9%. Host risk factors such as species, sex, breed, body condition score and physiological status (pregnancy) have shown statistically significant association with the overall prevalence of ectoparasites in small ruminants. The major identified ectoparasites were ticks, lice and fleas. Four genera of ticks (*Ambyloma, Boophilus, Rhipicephalus and Hyalomma*), two genera of lice (*Damalina and Linognathus*) and one genus of fleas (*Ctenocephalus*) were identified in this study. The high prevalence of ectoparasites in the study area affects the health, production and productivity of small ruminants. Hence, the high prevalence of ectoparasites prevailing in small ruminants in the study area needs serious attention.

Acknowledgements

The authors would like to acknowledge Haramaya University, College of Veterinary Medicine for providing laboratory facilities for the identification of the ectoparasites and the owners of the small ruminants for their cooperation and willingness during the sample collection process.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abebe, R., Tatek, M., Megersa, B. and Sheferaw, D., 2011. Prevalence of Small Ruminant Ectoparasites and Associated Risk Factors in Selected Districts of Tigray Region. *Global Veterinaria* 7 (5), 433-437.

Bekele, J., Tariku, M. and Abebe, R., 2011. External parasite infestations in small ruminants in Wolmera district of Oromia region, central Ethiopia. *Global Vet.*, 10, 518-523.

Chalachew, N., 2001. Study on skin diseases of cattle, sheep, and goat in and around wolayta soddo, Southern Ethiopia. DVM thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

CSA (Central Statistics Authority), 2004. Prevalence of Ectoparastes fauna of ruminants in Ethiopia, Addis Ababa.

Gatenby, M.R., 2002. Sheep, revised edition. The Tropical Agriculturalist, CTA and Macmillan,Wageningen, The Netherlands, pp: 128-135.

Hailu,Y., Tefera, Y., and Ayana, D., 2008. Ectoparasite prevalence's in sheep and goats in and around Wolita Soddo, Southern Ethiopia. *Reve. Med. Vet*., 159,450-454.

James, P., Carmichael, I., Pfeffer, A., Martin, R.. and O'Callaghan, M., 2002. Variations among Merino sheep in susceptibility to lice (*Bovicola ovis*) and association with susceptibility to trichostrongylid gastro-intestinal parasites. *Vet.Parasitol*., 103, 355-365.

Kassa, B., 2006. 'Cockle, mange and pox: Major threats to the leather industry in Ethiopia. Ethiopian leather industry: Perseverance towards value addition', Proceedings of the National Workshop, Addis Ababa, Ethiopia, December 14–15, 2006, pp. 71–92.

Kusiluka, L. and Kambarage, D.,1996. Disease of small ruminant, 1st ed., Scotland VET AID pp. 102-105.

MoARD, 2005. Mange, Lice and Sheep Ked control project in Amhara, Tigray and Afar regions, MoARD Animal Health Department, Addis Ababa.

Mohammed, M., 2000. Raw hides and skin improvement in Ethiopia Status and Challenges. In: Proceedings of the opportunities and Challenges of Goat Production in East Africa, A Conference held 10-12 Nov, 2000 at Debub University, Awassa, Ethiopia.

Mulugeta, Y., Yacob, T. and Ashenafi, H., 2010. Ectoparasites of Small Ruminants in three Selected Agro- Ecological sites of Tigray Region. *Trop. Anim. Health Prod*., 42(6), 1219-1224.

Radostits, O.M., Gay, C., Hinchcliff, K.W. and PConstable, P.D., 2007. A textbook of the diseases of cattle, sheep, goats, pigs and horses, 10 edition, Suanders, Edinburgh, London, pp: 1585-1612.

Sertse, T., 2004. Investigation in ectoparasites of small ruminants in selected sites of Amhara Regional State and their Impact on the Tanning Industry. MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre zeit, Ethiopia.

Steele, M.R., 1996. Goats. The Tropical Agriculturalist, CTA and Macmillan, Wageningen, pp: 79-86.

Teshome, W., 2002. Study on small ruminant skin disease in Sidama Zone, Southern Ethiopia. DVM thesis. Faculty of Veterinary Medicine, Addis Ababa University, Deber Zeit, Ethiopia.

Fentahun, T., Fasil, W., Mersha, C. and Malede, B., 2012. Prevalence of Ectoparasites on Small Ruminants in and Around Gondar Town. *American-Eurasian J. Sci. Res.,* 7 (3), 106-111.

Thrusfield, M., 1995. Veterinary epidemiology, 2nd ed. UK. Blackwell science pp, 182-189.

Urquhart, G.M., Armour, J Duncan, JL., Dunn, A.M. and Jennings, F.W., 1996. Veterinary Parasitology, 2nd edition. Blackwell Science Ltd., London, UK, pp: 89-205.

Walker, A.R.,Bouattour, A., Camicas, J.L., Estrada Pena, A., Horak, L.G., Latif, A.A. *et al*., 2003. Ticks of Domestic Animals in Africa. A Guide to Identification of Species. International Consortium on Ticks and Tick-borne Diseases (ICTTD-2), Bioscience Reports, University of Edinburgh, UK, pp: 221.

Zeleke, M. and Bekele, T., 2004. Species of ticks on camels and their seasonal dynamics in Eastern Ethiopia. *Trop. Anim Health Pro,*, 36, 225-231.

Hiko, Ethiop. Vet. J., 2019, 23 (1), 90-110
DOI https://dx.doi.org/10.4314/evj.v23i1.7 *Ethiopian Veterinary Journal*

Isolation, DNase-cross-Coagulase test and antimicrobial resistance test on *Staphylococcus* along beef abattoir line in Addis Ababa Ethiopia

*Adem Hiko**

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

Correspondence: E. mail: adex.2010ph@gmail.com

Abstract

Staphylococci are responsible for foodborne infection and intoxication with the spread of antibiotic resistance. The aims of the study were to investigate beef abattoir line contamination with *Staphylococcal*, to evaluate DNase test for alternative of the tube coagulase test, and to assess isolates drug resistance in Ethiopia. A total of 169 samples from slaughter environment, raw beef at inspection and at public supply along Addis Ababa Abattoir Enterprise line were examined for *Staphylococci.* The isolates were tested against DNase, tube plasma coagulase, and eight medicinal drugs. A total proportion of 35.5% (60/169) isolates with 13.6% *S. aureus* and 21.9% coagulase negative *Staphylococcus* (CNS) were observed. All sampling locations were found positive for *Staphylococcus* environmental samples ranged from 18.2% - 46.2% with no difference ($p > 0.05$) among locations. In raw beef, it was 23.5% at abattoir and 52.9% at butchers. Three (1.9%), 13 (7.7%) and 23 (13.6%) of locations were positive for *S. aureus* only, CNS only and both as a mixed, respectively. Of all 60 *Staphylococci* isolates, the DNase test and coagulase tests were in agreement for 56 isolates (21 for positive, 35 for negative) showing DNase test was strong agreement with the gold standard test (coagulase tests), kappa=0.86)). *S. aureus* was 38.3% but CNS was 61.7%. Resistant isolates were observed for trimethoprim (35.0%), polymyxin-B (33.7%), oxytetracycline (31.7%), trimethoprim-sulfamethoxazole (20.0%), chloramphenicol (8.3%), oxacillin (6.7%), and gentamycin (5.0%) but not for tetracycline. Thirty-one (51.7%) isolates were resistant for at least one drug with multiple drugs resistance (MDR) of three to six in 17 isolates. Contamination of all sampling locations with *Staphylococcus* including with resistant isolate to medically used drugs warrants the application of good hygienic practices along the abattoir line. Due to availability and cost effectiveness, DNase can be used as alternative to the gold standard, coagulase test, for diagnosis of *Staphylococcus*.

Key words: Abattoir line; beef; drug resistance; foodborne intoxication; *Staphylococci*

Introduction

Staphylococcus species are classified as classical pathogen, causing infections (Waldvogel, 1995; Lowry, 1998). But, heterogeneous group of *coagulase-negative Staphylococci* (CNS) those historically as being less or nonpathogenic (Becker *et al.,* 2014). They are differentiated based on their diagnostic reaction. Carriers are at higher risk of infection and they are presumed to be an important source of spread of *S. aureus* strains among individuals, to food and food production and processing chain (Muto *et al*., 2003; Kazakova *et al*., 2005; Miller and Diep, 2008). A fundamental biological property of *S. aureus* is the ability to asymptomatically colonize normal people. Approximately 30% of humans are asymptomatic nasal carriers of *S. aureus* (Kluytmans *et al*., 1997; Gorwitz *et al.,* 2008). Due to patient and procedure related changes, CNS also represent one of typical opportunistic major nosocomial pathogens having a substantial impact on human life and health, and also food associated saprophytes resulted in clinically manifested infections (Becker *et al.,* 2014).

The ideal identification of clinical *S. aureus* isolates requires a battery of tests and this is costly in resource limited settings. *S. aureus* identification was routinely done using either human or sheep plasma. Study was also conducted using mannitol salt agar and the deoxyribonuclease (DNase) test for improving the efficiency of the tube coagulase test in resource limited settings (Kateete *et al*., 2010). Indicating the absence of single phenotypic test (including tube coagulase) that can guarantee as reliable method for identification of *S. aureus,* Kateete *et al.* (2010) suggested the need for sequel testing of the isolates with Mannitol salt agar, DNase and Tube coagulase. On the other hand, the coagulase test is a golden standard method to differentiate *S. aureus* from coagulase negative *Staphylococcus (*Koneman *et al.,* 1997*).* Coagulase production can be detected using either slide coagulase test or the tube coagulase test. The tube coagulase test is better than the slide coagulase test in that it detects the secreted extracellular free coagulase which reacts with a substance in plasma called "Coagulase-Reacting Factor" (CRF) to form a complex clot (Koneman *et al.,* 1997; Winn *et al.,* 2006).

The extended use and misuse of antibiotics in agriculture, livestock farming and human medicine have rapidly facilitated the emerging of resistant bacteria strains including *Staphylococci* (Valsangiacomo *et al*., 2000). Antibiotic resistance leads to prolonged hospital stay and increased costs in terms of treatment (Kitara *et al*., 2011). Multidrug resistance is norm among *Staphylococci* pathogens (Lowy, 2003).

Study along milk production, Gizaw (2014) reported *Staphylococci* species at 47.1% from udder milk, 58% from tank milk of the selected dairy farms, 34.4% from cow bucket swab, 38% from farm tank swab, 38% from milkers hand and 70% from nasal swab of milkers. Similarly, prevalence of 38.5% from carcass swab, 37.8% from knife swab, 48.6% from slaughter line swab, 37.8% from abattoir worker hand and 46.7% nasal swab of butchers were reported in Ethiopia (Gizaw, 2014). Indicating the significance of drug resistance, pooled prevalence of methicillin resistant *S. aureus* was reported at rate of 32.5% from several report meta-analysis data in Ethiopia (Eshetie *et al*., 2016). On the other hand, structured abattoir line-based *Staphylococci* survey with DNase test were not yet conducted in Ethiopia. Therefore, the aims of the study were to investigate beef abattoir line contamination with *Staphylococcal* strain, to evaluate DNase test as alternative of the tube coagulase test and to assess drug resistance status of the isolates against medicinal drugs frequently used in Ethiopia.

Materials and Methodology

Abattoir lines description

The study was conducted at Addis Ababa Abattoir Enterprise (AAAE) located in Addis Ababa city. Multi-purpose cattle stocks purchased from extensive or semi-intensive management systems in different parts of the Ethiopia, transported to AAAE and slaughtered for human consumption were used. The AAAE has a capacity to slaughter up to 1,200 cattle in 8 hours with a staff of about 700 persons. After slaughter, carcasses were examined through routine meat inspection procedures. The raw beef, immediately or after a short cooling interval were loaded into meat transport trucks and delivered to city butcheries. Butcheries are mostly small open-stall shops, handling the meat at 20°C–27°C, which is the ambient temperature in Addis Ababa City.

Sampling and sample types

Samples were taken from December 2011 to April 2012 from the abattoir and beef retailer butchery. In the abattoir, different samples were taken from the operation environment. Sample of raw beef were also taken directly after quality inspection at abattoir. Following the beef identification number, same raw beef product were sampled at butchery houses located at Addis Ababa. A total of 169 samples from 10 sampling locations (Table 1) were taken. For swabs, a 50 cm^2 area was swabbed with sterilized gauze moistened with normal saline solution. Water (20 mL) sample was filled directly from the tap into sterile calibrated glass bottles. Meat samples (34 samples from AAAE and 34 samples from butcheries) were taken aseptically and placed in sterile stomacher bags. The number and types of samples were described within Table 1 with the results. Samples were immediately transported to Microbiology Laboratory, Akililu Lemma Institute of Pathobiology, Addis Ababa University (ALIPB-AAU), Ethiopia on the day of sampling using an ice box at +4°C.

Sample preparation

Each sample was aseptically taken. For pre-enrichment, buffered peptone water (Merck, Darmstadt, Germany) was used and the inoculate were incubated at 37°C for 18–20 hours. The first 1:10 dilution was homogenized with a Stomacher 400 (Seward Laboratory, London, UK) and incubated at 37°C for 18–20 hours to be used as pre-enrichment (Monttville *et al.,* 2012; USDA, 2012).

Staphylococcus isolation and characterization

A loopfull of pre-enrichment was inoculated on whole-hen-egg based Kranep Agar (Merck, Germany). One to five presumptive *Staphylococci* colonies were taken per-each sample. Colonies were screened using 3% hydrogen peroxide. The presumptive positive *S. aureus* colonies isolate was transferred to Standard-II Nutrient Agar (Merck, Germany) incubated at 37°C for 24 hr, stored at +4°C. The strain was exposed to deoxyribonuclease (DNase) and tube plasma coagulase test in parallel. An overnight cultured colony was inoculated in forms of line (Fig. 1) on DNase Agar (Merck, Germany) and incubated for 18-24 hr at 37°C. The culture was over flushed with 1 ml 1 mole/ml Hydrochloric acid. Based up on DNA digestion zone of clear transparence surrounding the culture, the strains were termed as DNase positive. Parallel to DNase test, tube plasma coagulase test (Bactident Merck, Germany) was done. The colony was inoculated in Brain Heart Infusion (BHI) (Merck, Germany) incubated at 37°C

for 18-24hr. A 0.5ml of rabbit plasma (Bactident Merck, Germany) was added in to sterile test tube followed by addition of 0.1ml of culture suspension. The mixture was incubated in 37°C water bath with 1hr interval up to 4 hr with registration of degrees of coagulation. Finally, the tubes were kept overnight at room temperature and re-examined.

Considered and used test protocol

In *Staphylococcus* identification, coagulase production can be detected using either the slide coagulase test or the tube coagulase test (Winn *et al*., 2006). The tube coagulase is better than the slide coagulase test in that it detects the secreted extracellular free coagulase which reacts with a substance in plasma called "Coagulase-Reacting Factor" to form a complex clot (Koneman *et al*., 1997; Winn *et al*., 2006). Thus, coagulase testing was used as gold standard for differentia between *S. aureus* and coagulase negative *Staphylococcus (*CNS*).* Control reactions included templates of *Staphylococcus aureus* ATCC 25923 (positive control), *Staphylococcus epidermidis* ATCC 12228 and nuclease free water (negative controls).

Ethical Considerations

Samples were also collected from adult personnel's hands who are working in the abattoir. Individuals involved on the sample were informed regarding the details of the research objectives. Hence, informed consent was collected.

Antimicrobial resistance test

The test was done on Mueller-Hinton agar (Oxoid, Hampshire, England) using Kirby Bauer disc diffusion criteria of the National Committee for Clinical Laboratory Standards (Bauer *et al*., 1996; CLSI, 2012). The following antimicrobial substances (Oxoid UK) were used: chloramphenicol (C 50 µg), gentamycin (CN 10 µg), oxytetracycline (OT 30 µg), oxacillin (OX 1 µg), polymyxin B (PB 300U), tetracycline (TE 10 µg), trimethoprim-sulfamethoxazole (STX 1.25/23.75 µg) and trimethoprim (W 5 µg (Oxoid, Hampshire, England). They were selected using the fact that they are used in health sectors of Ethiopia and their availability on the markets. The isolates were sub-cultured on Nutrient agar (Merck, Germany) and incubated at 37°C for 24 hrs. They were then inoculated into 5 ml of Brain Heart Infusion broth (BHI) (Merck, Germany) and again incubated for 1 hr at 37°C. The inoculum density was standardized using a 0.5 McFarland standard. Volume of 0.1 ml of standardized culture was

spread on Mueller-Hinton agar (Oxoid, Hampshire, England). The antibiotics disc was mounted and incubated at 37°C for 18–20 hours. Based on the diameter of inhibition zone, the results were recorded as susceptible, intermediate and resistant according to the rules of National Committee for Clinical Institute laboratory standards (CLSI, 2012).

Data analysis

Data were entered in to Microsoft Excel 2007® (Microsoft Corp., Redmond, USA) and analyzed using Excel, State 11, and SPSS version 20 (IBM Corp., Armonk, USA). Percentage and 95% confidence intervals (CI) of the percentage were used to demonstrate prevalence differences between and among the sampling occasions and types of samples. Using the coagulase test as gold standard (Winn et al., 2006; Koneman et al., 1997), the diagnostic test agreement (Kappa), sensitivity and specificity were also calculated. The cut-off Kappa value and interpretation was according to Viera, and Garrett (2005).

Results

Out of the total 169 samples, 35.5%; 95% CI 28.6%-42.9% were found positive for Staphylococcus. The prevalence ranges of 18.2% in hooks to 46.2% from personal hand swab with no difference ($p > 0.05$) were observed in environmental samples. In raw beef, it was 23.5% from when ready for distribution at abattoir and 52.9% in post distribution at butchers (Table 1).

Table 1: *Staphylococcus* by sampling locations and sample types along the Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample		Processing stages/position	Sampling location	Total № of sample	Total Staphylococcus	
					№ (%)	95% CI percentage.
Abattoir	Environment	Before beginning of operation	Personnel's hands	13	6 (46.2)	23.0-71.1
			Aprons	14	6 (42.9)	21.3-67.7
			Knives	13	4 (30.8)	12.8-58.1
			Hooks	11	2 (18.2)	5.5.-48.4
		Cleaning water	Tap water	12	3 (25.0)	9.1-53.8
		At carcass splitting	Rooms	17	5 (29.4)	13.3-53.5
		Refrigeration	Refrigerators	10	3 (30.0)	10.9-60.9
		Meat transport	Meat transport trucks	11	5 (45.5)	21.1-72.3
	Raw beef*	After washing for distribution	Beef inspection	34	8 (23.5)	12.5-40.1
		Sub total		135	42 (31.1)	23.4-39.6
Butchers		Butchers, 6-8 hours post delivery	Beef for consumption	34	18 (52.9)	35.1-70.2
Total				169	60 (35.5)	28.6-42.9

Note: CNS = Coagulase negative Staphylococci; CI = Confidence interval; Mid Pexct. = Mid prevalence exact; * Raw beef at abattoir

From a total sample, 13.6% S. aureus and 21.9% CNS were observed (Table 2). Among environmental samples, S. aureus ranged from 0% on knives to 36.4% in meat transport trucks while CNS ranged from 0% on hooks to 46.2% on personnel's hands swabs. In raw beef, S. aureus was 8.8% at ready for distribution but raised to 20.6% post-delivery at public supply. Similarly, CNS was 14.7% at ready for distribution but raised to 32.4% post-delivery at public supply.

Table 2: Distribution of *S. aureus* and CNS by sampling locations and sample types along Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample		Sampling location	TN№ of sample	*S. aureus* № (%)	CNS № (%)
Abattoir	Environment	Personnel's hands	13	3 (23.1)	3 (23.1)
		Aprons	14	2 (14.3)	4 (28.6)
		Knives	13	0	4 (30.8)
		Hooks	11	2 (18.2)	0
		Tap water	12	1 (8.3)	2 (16.7)
		Rooms	17	2 (11.8)	3 (17.6)
		Refrigerators	10	2 (20.0)	1 (10.0)
		Meat transport trucks	11	1 (9.1)	4 (36.4)
	Raw beef*	Beef inspection	34	3 (8.8)	5 (14.7)
	Subtotal		135	16 (11.9)	26 (19.3)
Butchers		Beef for consumption	34	7 (20.6)	11 (32.4)
Total			169	23 (13.6)	37 (21.9)

* Raw beef at abattoir

Mixed occurrence of *S. aureus* and CNS by sampling locations was shown in Table 3. *S. aureus* only, CNS only and co-occurrence were 1.9%, 7.7% and 13.6% respectively. Except on knives and hooks, mixed occurrences of *S. aureus* and CNS were observed in all other sampling locations.

Table 3: Mixed occurrence of *S. aureus* and CNS by sampling locations and sample types along Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample		Sampling location	Total № of sample	S. aureus only № (%)	CNS only № (%)	Mixed isolation № (%)
Abattoir	Environment	Personnel's hands	13	0	0	3 (23.1)
		Aprons	14	0	2 (14.3)	2 (14.3)
		Knives	13	0	4 (30.8)	0
		Hooks	11	2 (18.2)	0	0
		Tap water	12	0	1 (8.3)	1 (8.3)
		Rooms	17	0	1 (5.9)	2 (11.8)
		Refrigerators	10	1 (10.0)	0	1(10.0)
		Meat transport trucks	11	0	1 (9.1)	4 (36.4)
	Raw beef*	Beef inspection	34	0	2 (5.9)	3 (8.8)
	Subtotal		135	3 (2.2)	9 (6.6)	16 (11.9)
Butchers		Beef for consumption	34	0	4 (11.8)	7 (20.6)
Total			169	3 (1.9)	13 (7.7)	23 (13.6)

* = Raw beef at abattoir

The coagulase test was used as gold standard. Of all 60 isolates exposed to DNase (Figure 1) and coagulase tests, 21 were positive for both tests whereas 35 were negative for both test reactions showing (Kappa = 0.86; 95% CI = 0.73-0.99**)** Sensitivity = 91.3% and Specificity = 94.6%) (Table 4). Out of the total 60 isolates, 23 (38.3%) and 37 (61.7%) isolates were coagulase positive **Staphylococcus** (CPS) and coagulase negative **Staphylococcus** (CNS), respectively.

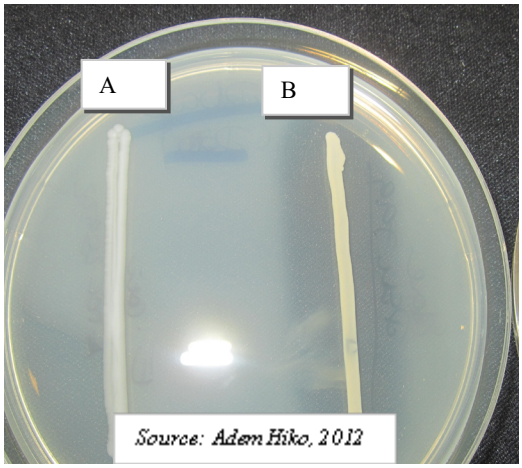


Figure 1: DNase test reaction of isolated *Staphylococcus*: A) DNase negative, B) DNase positive (Clear zone of DNA digestion).

Table 4: DNase-cross-Coagulase test reactions of *Staphylococcal* isolates from the Addis Ababa Abattoir Enterprise beef abattoir line

Diagnostic test		№ of tube coagulase		Total № (%)
		Positive	Negative	
№ of DNase	Positive	21*	2**	23 (38.3)
	Negative	2***	35****	37 (61.7)
Total № (%)		23 (38.3)	37 (61.7)	60 (100)

Note: True positives* = 21; False positives** = 2; False negatives***= 2; True negatives****=35; Almost perfect agreement (Kappa= 0. 86 (95% CI = 0.73-0.99)); Sensitivity = 91.3%; Specificity = 94.6%;

Antimicrobial Resistance test

The 60 Staphylococcus isolates were subjected to 8 types of antibiotics for antimicrobial resistance test. Out of the total isolates, 35.0% were resistance to trimethoprim, 33.7% for polymyxin B, 31.7 % for oxytetracycline, 20.0% for trimethoprim-sulfamethoxazole, 8.3% for chloramphenicol, 6.7% for oxacillin, 5.0% for gentamycin and no resistance was observed for tetracycline (Table 5).

Table 5: Drug resistance profiles of the Staphylococcal isolates from the Addis Ababa Abattoir Enterprise beef abattoir line.

Drugs used	S. aureus (n = 23)			CNS (n = 37)			Total (N = 60)		
	S. № (%)	I. № (%)	R. № (%)	S. № (%)	I. № (%)	R. № (%)	S. № (%)	I. № (%)	R. № (%)
TE	23 (100)	0	0	37 (100)	0	0	60 (100)	0	0
CN	20 (86.9)	1 (4.3)	2 (8.7)	36 (97.3)	0	1 (2.7)	56 (93.3)	1 (1.7)	3 (5.0)
OX	20 (86.9)	0	3 (13.1)	32 (86.5)	4 (10.8)	1 (2.7)	52 (86.6)	4 (6.7)	4 (6.7)
C	19 (80.6)	1 (4.3)	3 (13.1)	35 (94.6)	0	2 (5.4)	54 (90.0)	1 (1.7)	5 (8.3)
SXT	12 ()52.2	1 (4.3)	10 (43.5)	34 (91.9)	1 (2.7)	2 (5.4)	46 (76.7)	2 (3.3)	12 (20.0)
OT	10 (43.5)	0	13 (56.5)	30 (81.1)	1 (2.7)	6 (16.2)	40 (66.7)	1 (1.7)	19 (31.7)
PB	6 (26.1)	0	17 (73.9)	34 (91.9)	0	3 (8.1)	40 (66.7)	0	20 (33.7)
W	7 (30.4)	2 (8.7)	14 (60.9)	26 (70.3)	4 (10.8)	7 (18.9)	33 (55.0)	6 (10.0)	21 (35.0)

Note: C = Chloramphenicol; CN = Gentamycin; TE = Tetracycline; OT = Oxytetracycline; OX = Oxacillin; PB = Polymyxin B; SXT = Trimethoprim-Sulfamethoxazole; W = Trimethoprim

Antimicrobial resistance at different sampling sites

Two (5.9%) to 12 (35.3%) resistant *Staphylococcus* isolates from abattoir environmental samples was observed against all drugs used in this study. Similarly, resistant isolates to drugs used in this study were distributed on beef at abattoir and butchery. Exceptions are oxacillin resistant isolates were not observed on beef from abattoir and butchery while gentamycin resistant isolates were not observed in beef at abattoir (Table 6).

Table 6: Distribution of resistant *Staphylococcus* isolates by origin of sample at Addis Ababa Abattoir Enterprise beef abattoir line.

Origin of sample	№ test	№ (%) resistant Staphylococcus isolates*						
		CN	OX	C	SXT	OT	PB	W
Environment**	34	2 (5.9)	4 (11.8)	12 (35.5)	7 (20.6)	11 (32.4)	11 (32.4)	12 (35.3)
Beef at abattoir	8	0	0	3 (37.5)	3 (37.5)	2 (25.0)	5 (62.5)	3 (37.5)
Beef at butcher	18	1 (5.6)	0	6 (33.3)	2 (11.1)	6 (33.3)	4 (22.2)	3 (37.5)
Total	60	3 (5.0)	4 (6.7)	21 (35.0)	12 (20.0)	19 (31.7)	20 (33.3)	21 (35.0)

Note: C = Chloramphenicol; CN = Gentamycin; TE = OT = Oxytetracycline; OX = Oxacillin; PB = Polymyxin B; SXT = Trimethoprim-Sulfamethoxazole; W = Trimethoprim; * = Tetracycline resistant isolates were not observed; ** = Abattoir Environment*

Higher resistant *S. aureus* the CNS were observed among the used drugs (Table 7). Two (15.4%) to ten (76.9%) resistant *S. aureus* isolates to chloramphenicol, gentamycin, oxytetracycline, oxacillin, polymyxin B, trimethoprim-sulfamethoxazole and trimethoprim were observed at abattoir environment (Table 7). On the other hand, resistant *Staphylococcus* isolates to chloramphenicol, gentamycin and oxacillin was not observed on beef both at abattoir and butchery. However, regardless of the number, high percentages of resistant *Staphylococcus* isolates to oxytetracycline, polymyxin B, trimethoprim-sulfamethoxazole and trimethoprim were observed on beef at butchery than at abattoir. With regards to CNS, variable types of resistant isolates to the used drugs were observed at each sample origin.

Table 7: Distribution of resistant *Staphylococcus strain* isolates by origin of sample at Addis Ababa Abattoir Enterprise beef abattoir line.

Staphylo-coccus strain	Origin of sample	№ test	№ (%) resistant Staphylococcus isolates*						
			CN	OX	C	SXT	OT	PB	W
S. aureus	Environment**	13	2 (15.4)	3 (23.1)	3 (23.1)	7 (53.9)	8 (61.5)	10 (76.9)	10 (76.9)
	Beef at abattoir	3	0	0	0	2 (66.7)	2 (66.7)	3 (100)	2 (66.7)
	Beef at butcher	7	0	0	0	1 (14.2)	3 (42.9)	4 (57.1)	2 (28.6)
	Total	23	2(8.7)	3 (13.1)	3 (13.0)	10 (43.5)	13 (56.5)	17 (73.9)	14 (60.9)
CNS	Environment**	21	0	1 (4.8)	0	0	3 (14.3)	1 (4.8)	2 (9.5)
	Beef at abattoir	5	0	0	1 (20.0)	1 (20.0)	0	2 (40.0)	1 (20.0)
	Beef at butcher	11	1 (9.1)	0	1 (9.1)	1 (9.1)	3 (27.3)	0	4 (36.4)4
	Total	37	1 (2.7)	1(2.7)	2 (5.4)	2 (5.4)	6 (16.2)	3 (8.1)	7 (18.9)

Note: C = Chloramphenicol; CN = Gentamycin; OT = Oxytetracycline; OX = Oxacillin; PB = Polymyxin B; SXT = Trimethoprim-Sulfamethoxazole; W = Trimethoprim; * = TE resistant isolates were not observed; ** = Abattoir Environment*

Resistance pattern

Out of the tested 60 isolates, 31 (51.7%) were resistant to one drug to multiple of six drugs (Table 8). Ten isolates for single drug, four for two drugs but the remaining 17 for multiple of three to six drugs resistance (MDR) were observed. Higher isolates of 5 were found resistant to four dug combination of oxytetracycline-polymyxin B-trimethoprim-sulfamethoxazole-trimethoprim (OT-PB-SXT-W).

Table 8: Single to multiple of six drug resistant patterns of the isolates at Addis Ababa Abattoir Enterprise beef abattoir line.

Resistant isolates pattern	Drugs of resistant	No. of *S. aureus*	No. of CNS	No. of Total
Single drug resistant	C	0	1	1
	OT	0	3	3
	OX	0	1	1
	PB	2	1	3
	W	0	2	2
	Subtotal* № *(%)	***2 (11.1)***	***8 (61.5)***	***10 (32.3)***
Two drugs resistant	C-W	0	1	1
	OT-PB	2	0	2
	PB-W	1	0	1
	Subtotal* № *(%)	***3 (16.7)***	***1 (7.7)***	***4 (12.9)***
Three drugs resistant	C-OT-W	0	1	1
	OT-SXT-W	0	1	1
	OT-PB-W	2	1	3
	PB-SXT-W	1	1	2
	Subtotal* № *(%)	3 (16.7)	4 (30.8)	7 (22.6)
Four drugs resistant	OT-PB-SXT-W	5	0	5
	OX-PB-SXT-W	1	0	1
	***Subtotal* №. (%)**	***6 (33.3)***	***0***	***6 (19.4)***
Five drugs resistant	C-CN-OT-SXT-W	1	0	1
	OT-OX-PB-SXT-W	1	0	1
	C-OT-PB-SXT-W	1	0	1
	Subtotal* № *(%)	***3 (16.7)***	***0***	***3 (9.7)***
Six drugs resistant	C-CN-OT-OX-PB-W	***1 (5.6)***	***0***	***1 (3.2)***
Total № (%)	31	18 (100)	13 (100)	31 (100)

Discussion

Prevalence of *Staphylococcus*

Meat is an important vehicle for the transfer of antibiotic resistances from animals to humans, and antimicrobial resistance has always been a major concern for nosocomial infections in hospital environments. Such transfer can occur in three ways: by means of antibiotic residues in food, through the transfer of resistant food borne pathogens, or through the ingestion of resistant parts

of the original food microflora and resistance transfer to pathogenic microorganisms (Kruse and Sorum, 1994; Klein, 1999; Teuber, 1999; Mayrhofer *et al*., 2004). The present overall prevalent of 35.5% of *Staphylococcus* is slightly lower than the 42.8% total *Staphylococcal* reported by Gizaw (2014). However, there is similarity in prevalence of *S. aureus* at hooks (18.2%) and personnel hand swab (46.2%). The raising *Staphylococcal* load from 23.5% at ready for distribution at abattoir to 52.9% butchers house indicated the high risk of beef contamination by *S. aureus* carriers human being and from the beef handling environment like transporting trucks (45.5%) along the meat value chain (Gudeta, 2012). Asymptomatic nasal carriers of *S. aureus* (Kluytmans *et al.,* 1997; Gorwitz *et al.,* 2008; Gizaw, 2014) as a normal flora with human an important source of spread of *S. aureus* to food and food production and processing chain were also indicated by Miller and Diep (2008), Kazakova *et al.* (2005) and Muto *et al.* (2003). This was supported by 46.2% *Staphylococcal* isolation on personnel's hands swabs observed from the current study.

The present findings of *S. aureus* (8.8%) and CNS (14.7%) were lower than the *S. aureus* prevalence 38.5% from carcass swab (Gizaw, 2014), 28.57% from poultry and 29.41% from beef, 23.86% *S. aureus* from raw meat (Pesavento *et al.,* 2007) but similar was 15.15% from pork (Pesavento *et al.,* 2007). But the prevalence on ready for distribution and the 20.6% *S. aureus* and 32.4% CNS were similar with reports of Gizaw (2014). This variation could due to difference in study area and sources of sample. Moreover, the present concomitant raising of *S. aureus* (8.8%) and CNS (14.7%) at ready for distribution to raise to 20.6% and 32.4% respectively at post-delivery at public supply could be due to further contamination of the beef product at postharvest stages followed by growth of microbial. These indicate the increasing in rate of contamination and multiplication of *Staphylococcal* on the carcasses and along the beef handling stage. Rani *et al.* (2017) suggested meat distribution stage as the most critical period where the quality can easily be compromised. During distribution, pathogenic and spoilage micro-organisms may grow (Rani *et al*., 2017).

DNase and Coagulase test

Coagulase testing was used as gold standard for differentia between S. aureus and coagulase negative Staphylococcus (CNS) (Winn et al., 2006). Clinical S. aureus isolation requires a cost effective and better tests in resource limited developing countries (Kateete et al., 2010) where tube plasma coagulase test is confirmatory. However, as an alternative diagnostic tool, DNase test can assist

or replace it. The present finding showed almost perfect agreement (Kappa = 0.86, 95%CI = 0.73-0.99) between the DNase and tube plasma coagulase tests in screening S. aureus from the CNS. According to Viera and Garrett (2005), almost perfect test agreement ranges from 0.81-0.99.

The present 91.3% sensitivity was found in agreement and lower than 94-100% previous findings reported by other investigators (Tager **et al.,** 1948, Orth **et al.,** 1971; Oranusi and Umoh, 2006) but higher than the 75-76% sensitivity reported by Kateete **et al. (**2010) using tri-combination test (human or sheep plasma/Mannitol salt agar/DNase). The differences could be due to difference in the test method where by Kanep agar and rabbit plasma were used in this study. The present 94.6% specificity is found lower than the 100% reported by Kateete **et al.**, (2010) whose used tri-combination test both by (human plasma/Mannitol salt agar/DNase). "The two DNase negative **S. aureus**" in the context of tube coagulases the gold standard, DNase negative but tube coagulase**s** positive are false negatives isolates. They may probably be MRSA isolates which are reported to react weakly. Presence of such rare coagulase negative **S. aureus** strains has been reported by Koneman **et al.** (1997) which could weakly coagulase reacting strain termed as MRSA or **Staphylococcus schleiferi subsp coagulans.** (Kateete **et al.,** 2010). Again, Rao **et al**. (2002) also reported DNase negative **S. aureus.** On the other hand, the present two isolates with DNase positive but tube coagulase negative (i.e. false positive isolates) were presume **Staphylococcus schleiferi subsp. coagulans.** Kateete **et al. (**2010) also observed isolates with such property with similar assumption in Uganda.

Drug resistance profile of Staphylococcal species

The rapidly increasing in the number of antimicrobial resistant bacteria agents could be due to the extended use and misuse of antibiotics in livestock farming and human diseases treatment). *Staphylococcal* species (Kitai *et al*., 2005; Becker *et al.,* 2014), have shown a considerable increase in resistance against most antibiotics (Valsangiacomo *et al*., 2000) with the consequence of prolonged hospital stay and increased costs of treatment (Kitara *et al*., 2011). Tetracycline resistant isolate was not observed in this study but 62.5% were reported from milk (Gizaw, 2014). Again, unlike the present finding, 19.0% *S. aureus* were resistant to tetracycline with 8.3%, 20.0% and 25.0% from poultry, beef and pork were reported (Pesavento *et al*., 2007). This could be due to the difference among the sources of isolates and geographical location. On

the other hand, the 31.7% resistant isolate to oxytetracycline observed in this study could be from frequently use of this in Ethiopia in animal's treatment (DACA, 2009) which might develop drug resistance. Present finding showed higher resistant *S. aureus* (43.5%) than CNS (5.4%) to co-trimoxazole indicating greater risk of resistance development of the pathogenic strain than the less pathogenic group to commonly used drug. The current 20.0% resistance to co-trimoxazole was lower than the resistant *S. aureus* from outpatients (53.6%) and inpatients (44.7%) (Kitara *et al.*, 2011). The overall 6.7% oxacillin resistant *Staphylococci* with *S. aureus* (13.1%) and CNS (2.7%) were lower than the 35.7% resistant *S. aureus* isolates (Pesavento *et al.,* 2007). This finding is still lower than the 66.7% from poultry and 30.0% from pork oxacillin resistant *S. aureus*. But the present oxacillin resistant *S. aureus* was similar with the 10.0% reports from beef meat items (Pesavento *et al.,* 2007). The current over all 5.0% resistance to gentamycin was similar with a pooled 9.5% resistant *S. aureus* reported by Pesavento *et al.* (2007) but lower than the 16.7%, 10.0% and 5.0% from poultry, beef and pork (Pesavento *et al.* (2007) respectively.

The present 15.4% to 76.9% resistant *S. aureus* to chloramphenicol, gentamycin, oxytetracycline, oxacillin, polymyxin B, trimethoprim-sulfamethoxazole and trimethoprim were observed at abattoir environment could be due to contamination of the abattoir environment from animals, or from carrier humans particularly from abattoir workers harboring resistant strain. Evidence that transfer of antimicrobial resistance from food-producing animals to humans directly via the food chain as route of spread were documented (Wooldridge, 2012). The absence of resistant *Staphylococcus* isolates to chloramphenicol, gentamycin and oxacillin on beef both at abattoir and butchery could be due to the fact that these drugs are not used in animal treatment in Ethiopia where no development of resistant strain. The occurrence of one or more resistant almost along the studied beef line location indicates the contamination of environmental sites which concerned as one routes of resistant isolate circulation via contact. Contaminated environmental sites were suggested as pathway for the transfer of resistant isolate in meat (Wooldridge, 2012; Bengtsson-Palme *et al.,* 2018). Moreover, transfer of resistant genetic elements between bacteria in mixed populations as a cross resistance to drugs of similar generic groups could make complex potential routes of resistant isolate spread. Similar suggestions were given (Wooldridge, 2012; Bengtsson-Palme *et al.,* 2018).

Total of 51.7% resistant isolates to one drug to multiple of six drugs consisting as multiple drugs resistance (MDR) were observed. A large proportion

(89.3%) from food line (Gizaw, 2014), 53.7% from hospital cases (Gizachew *et al.,* 2015) were showed MDR in Ethiopia. The presences of nasal carriage of resistance staphylococcus were also reported by Tewodros and Gedebou (1984) in Ethiopia. Like the present 51.7% MDR isolation, Pesavento *et al.* (2007) also reported 30.95% resistant isolates to at least three antibiotics in indicating significance resistance among these pathogens.

Conclusion and recommendation

The present finding indicates increased contamination of meat product along handling line with both by *S. aureus* and CNS with more than half percent of the isolates being resistant to single to multiple drug resistant. Thus, this finding warrants the application of good hygienic practices along the beef abattoir line with control of drug resistance. The DNase test showed strong agreement with tube coagulase test with low false positive and negative isolates indicated the possible alternative use of these diagnostic tests for screening staphylococcal isolates in resource-limited settings like Ethiopia.

Acknowledgement

Cooperation of Addis Ababa Abattoir Enterprise staff in providing the requested sample on time was highly appreciated. Prof. Fries Reinhard was highly appreciated for microbiologic media support and Prof. Gobena Ameni provided laboratory equipment. Aklilu Lemma Institute of Pathobiology Addis Ababa University was appreciated for provision of laboratory space.

Conflict of interest

There is no conflict of interest to declare

References

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck. M., 1966. Antibiotic susceptibility testing by standard single disc method. *Am. J. Clin. Patholo*., 45(4), 493-496.

Becker, K., Heilmann, C., Peters, G., 2014. Coagulase-Negative Staphylococci, *Clin. Microbiol. Rev.,* 27 (4), 870–926. doi:10.1128/CMR.00109-13.

Bengtsson-Palme, J., Kristiansson, E., Larsson, D.G.J., 2018. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiol. Rev*., 42(1), fux053. doi: 10.1093/femsre/fux053.

CLSI, 2012. 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 (DACA), 2009, Addis Ababa, Ethiopia.

Eshetie, S., Tarekegn, T., Moges, F., Amsalu, A., Birhan, W., Huruy. K., 2016. Methicillin resistant *Staphylococcus aureus* in Ethiopia: a meta-analysis. *BMC. Infect. Dis.*, 16, 689. doi 10.1186/s12879-016-2014-0.

Gizachew, M., Abdella, H., Tiruneh, M., 2015. Antimicrobial Susceptibility Patterns of *Staphylococcus aureus* at the University of Gondar Tertiary Hospital, Northwest Ethiopia: A Retrospective Cross Sectional Study. *J. Bacteriol. Parasitol.*, 6 (3), 228. doi:10.4172/2155-9597.1000228.

Gizaw, F., 2014. Staphylococcus: Epidemiology and its drug resistance in cattle, food Chains and humans in central Ethiopia, MSc thesis. Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia.

Gorwitz, R.J., Kruszon-Moran, D., McAllister, S.K., McQuillan, G., McDougal, L.K., Fosheim, G.E., Jensen, B.J., Killgore, G., Tenover, F.C., Kuehnert, M.J., 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States 2001–2004. *J. Infect. Dis.*, 197(9), 1226–1234. doi: 10.1086/533494.

Gudeta, B., 2012. Optimizing logistic chain of animal transport and meat distribution: studies on livestock markets and abattoirs in Addis Ababa City. MSc thesis, Addis Ababa University, Civil Engineering/Road and Transport Engineering Stream, Addis Ababa, Ethiopia.

Kateete, D.P., Kimani, C.N., Katabazi, F.A., Okeng, A., Okee, M.S., Nanteza, A., Joloba, M.L., Najjuka, F.C., 2010. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Ann. Clin. Microbiol. Antimicrob.*, 9, 23. doi: 10.1186/1476-0711-9-23.

Kazakova, S.V., Hageman, J.C., Matava, M., Srinivasan, A., Phelan, L., Garfinkel, B., Boo, T., McAllister, S, Anderson, J., Jensen, B., Dodson, D., Lonsway, D., McDougal, L.K., Arduino, M., Fraser, V.J., Killgore, G., Tenover, F.C., Cody, S., Jernigan, D.,B., 2005. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. *N. Engl. J. Med.*, 352(5), 468–475. doi: 10.1056/NEJMoa042859.

Kluytmans, J., van Belkum, A., Verbrugh, H., 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol. Rev.*, 10(3), 505–520.

Koneman, E.W.A.S., Janda, W.M., Schreckenberger, P.C., Winn, W.C., 1997. The Gram-positive cocci: *Staphylococci* and related organims. In Color Atlas and Textbook of Diagnostic Microbiology. Philadelphia: Lippincott-Raven Koneman EW, 5, 551-576.

Lowry, F.D., 1998. *Staphylococcus aureus* infections. *New. Engl. J. Med*., 339, 520-532. doi: 10.1056/NEJM199808203390806.

Lowy, F.D., 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J. Clin. Invest.,* 111(9), 1265–1273. doi: 10.1172/JCI200318535.

Miller, L.G., Diep, B.A., 2008. Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. *Clin. Infect. Dis*., 46, 752–760. doi: 10.1086/526773.

Monttville, T.J., Matthews, K.R., Kniel, K.E., 2012. Food microbiology: An introduction, 3rd edition. Washington, ASM Press 570 p.

Muto, C.A., Jernigan, A.J., Ostrowsky, B.E., Richet, H.M., Jarvis, W.R., Boyce, J.M., Farr, B.M., 2003. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and enterococcus. *Infect. Control Hosp. Epidemiol*., 24(5), 362–86. doi: 10.1086/502213.

Oranusi, S.G, Umoh, V.J, 2006. Toxicity test and bacteriophage typing of *Staphylococcus aureus* isolates from food contact surfaces and foods prepared by families in Zaria, Nigeria. *Afr. J. Biotech.,* 5(4):362-365.

Orth, D.S., Chugg, L.R., Anderson, A.W., 1971. Comparison of Animal Sera for Suitability in Coagulase Testing. *Appl. Environ. Microbiol.,* 21(3), 420-425. PMC: 377196.

Pesavento, G., Ducci, B., Comodo, N., Nostro, A., 2007. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). *Food Control*, 18(3), 196–200. doi. org/10.1016/j.foodcont.2005.09.013

Rani, Z. T., Hugo, A., Hugo, C. J., Vimiso, P., Muchenje, V., 2017. Effect of post-slaughter handling during distribution on microbiological quality and safety of meat in the formal and informal sectors of South Africa: A review. *S. Afr. J. Anim. Sci*., 47 (3), 255-267.

Rao, J.G., Qamruddin, A.O., Hassan, I.A., Burnie, J.P., Ganner, M., 2002. Cluster of clinical isolates of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA) with a negative deoxyribonuclease (DNase) test-implications for laboratory diagnosis and infection control. *J. Hosp. Infect*., 51(3), 238-239.

Tager, M., Hales, H.B., 1948. The Experimental Production of Antibodies to Staphylocoagulase. *J Immunol.*, 60 (4), 475-485. PMID: 18106198

Tewodros, W., Gedebou, M., 1984. Nasal carrier rates and antibiotic resistance of *Staphylococcus aureus* isolates from hospital and non-hospital populations, Addis Ababa. *Trans. R. Soc. Trop. Med. Hyg.,* 78 (3), 314-318. PMID: 6464129.

USDA, 2012. Introduction to the microbiology of food processing. Small plant news guidebook series United States Department of Agriculture (USDA) Food Safety and Inspection Service. August 2012, pp. 1-64.

Viera, A.V., Garrett, J.M., 2005. Understanding Interobserver Agreement: The Kappa Statistic, *Fam. Med*., 37(5), 360-363.

Waldvogel, F.A., 1995. *Staphylococcus aureus* (including toxic shock syndrome). In: Mandell, G.L., Bennett, J.E., Dolin, R., editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, 4th Edn. New York, Churchill Livingstone, pp. 1754 – 1777.

Winn, W., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P., Woods, G., 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Edition, Lippincott Williams and Wilkins, New York

Wooldridge, M. 2012. Evidence for the circulation of antimicrobial-resistant strains and genes in nature and especially between humans and animals. *Rev.Sci. Tech*., 31(1), 231-247.

Abera and Mulate, Ethiop. Vet. J., 2019, 23 (1), 111-130
DOI https://dx.doi.org/10.4314/evj.v23i1.8 *Ethiopian Veterinary Journal*

Ethno-veterinary medicine: A potential alternative to animal health delivery in Wolmera district, Oromia Region, Ethiopia

*Yohans Abera [1] and Belay Mulate [1] **

1 School of Veterinary Medicine Wollo University P.O.Box 1145, Ethiopia

**Corresponding author: Belay M, Email: dorogibir@ gmail.com*

Abstract

This survey was conducted from December 2016 to March 2017 in Wolmera district of Oromia Region, Ethiopia to document indigenous knowledge of the community on plants used in ethno-veterinary medicine. From a total 80 respondents from eight peasant associations, 48 respondents were selected purposely by the researcher alone while the rest 32 were selected purposely by the help of local elders in the study area. Majority of respondents 63(87.27%) were male and the remained 17 (12.72%) were females. Most of the respondents 55(68.75%) were used plants to treat livestock ailment, and 50 (91%) acquired the knowledge from their parents and elders of blood relationship. Twenty- five medicinal plant species of veterinary importance classified into 22 families were documented. From identified plant families, asteraceae, euphorbiacea, solonaceae and polygonaceae were represented by 2 species each. The remaining (17 families) were represented by single plant species. Leaves were the most frequently utilized plant part (68%) and oral route (80%) was the most common route of administration, whereas concoction (60%) accounted the major form of preparation of herbal remedies. Determination of the dose is done by using different household utensils and also by using their own hand. Anthrax, black leg, ecto and endo parasites, rabies, foot and mouth disease, bloat, and colic were among the fifteen different livestock diseases or syndromes treated by traditional practitioners. Fifty-five out of 80 respondents who participated in this survey have an awareness on ethnoveterinary practice but plant parts used, dose and routes of administration were varies among the traditional practitioners. Therefore, we strongly recommended for detail scientific and pharmacological evaluations (efficacy, safety, mode of delivery and dosage) of the identified plant species in future use.

Key words*:* Aliments; Endogenous knowledge*;* Ethno veterinary*;* Medicinal plants

Introduction

Absence or inaccessibility to modern veterinary services and high cost of modern drugs, as well as failure in their efficacy, a large proportion of farmers rely on traditional medicine (herbal remedies) to keep their livestock healthy and productive for several decades (Kaur *et al*., 2011). In Ethiopia, due to huge potential of medicinal plants, traditional herbal medicine is an integral part of the local culture and used widely for the treatment of human and livestock aliments (Giday and Teklehaymanot, 2013).

Ethno-veterinary knowledge like other traditional knowledge is transferred from generation to generation through the word of mouth (oral tales) with great secrecy through family members, horizontally by exchange through peers, or diagonally through traditional healers to learners (Yirga *et al.,* 2012). This knowledge may disappear because of rapid socio-economic and technological changes. Moreover, environmental degradation, agricultural expansion (cultivation of marginal lands) and urbanization warranting urgent need to document and preserve the indigenous knowledge (Kalayou *et al*., 2012; Khan *et al*., 2012)

For the sake of documentation of endogenous knowledge regarding ethnoveterinary medicinal plants, several studies have been conducted throughout the world such as in South Africa (Vander and Swan, 2001) , in Zimbabwe(Tafara and Taona, 2004*)*, in China (Shen *et al*.,2010*)*,in Pakistan *(*Deeba *et al.,* 2009) and in India (Somvanshi,2006). Similarly, in Ethiopia, studies have been conducted on plant remedies which are used extensively by animal owners to treat livestock diseases (Giday and Ameni, 2003; Hunde *et al*., 2004; Kebu *et al.,* 2004; Sori *et al.,* 2004; Yineger *et al*.,2007; Yineger *et al*., 2008; Yigezu *et al.,* 2014*).* However, the effort is still quite insignificant when compared to the undocumented ethnoveterinary plant lore of the country. Therefore, the present study was conducted to identify and document plants used in ethnoveterinary practices along with livestock ailments treated by farmers in Wolmera district of Oromia Region, Ethiopia.

Materials and Methods

Study area

This study was conducted from December 2016 to March 2017 in eight selected peasant associations (PAs) of Wolmera District of Oromia Region, Ethiopia. The district is located at a distance of 33 km West of the capital, Addis Ababa at latitude 9º 3' N and 38º 30' E longitude (Fig 1). The district is largely a highland area. The highest point in this district is mount *Wechecha* with an elevation of 3191 m.a.s.l, located in the southern part of the district. The Menagesha National forest (2500 hectares in size) covers the southern and western slopes of this mountain (Briggs, 2002). The average annual rainfall of the district is 1014 mm. The maximum and minimum temperatures of the area are 22.3ºC and 6.16ºC, respectively; and the area experiences a bimodal rainfall patterns with a short rainy season which occurs from March to May and long rainy season which starts at the end of June and ends at early November. Oromiffa is spoken as a first language by 70.1% of the residences, whereas 24.22% spoke Amharic and the remaining 5.68% spoke other languages. Crop-livestock farming system is being practiced in the district and the dominant livestock raised in the district is cattle, small ruminants and equines in their decreasing order (CSA, 2015).

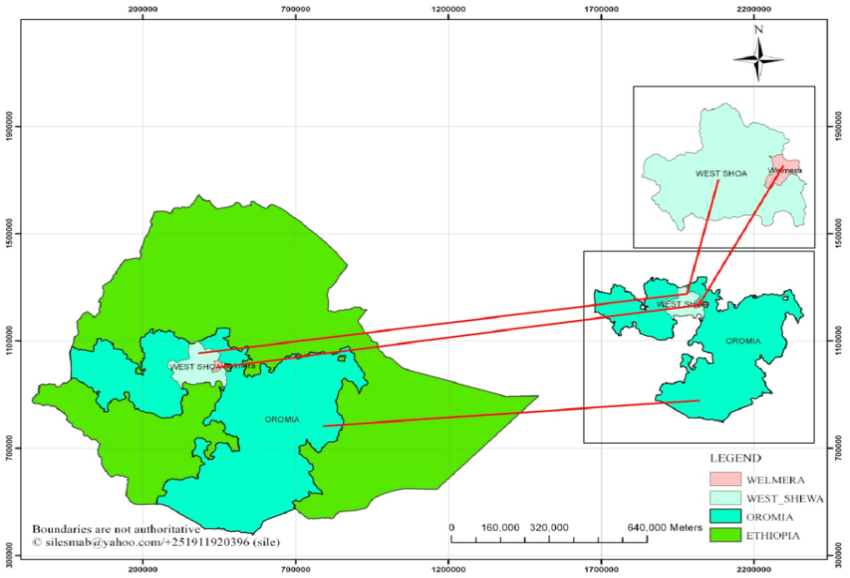


Figure 1. Map of the study district

Study design

A community based cross-sectional study design supported by questionnaire and close observation was conducted to collect Ethno-veterinary knowledge on medicinal plants in the selected peasant associations. A semi-structured questionnaire was prepared and administered to 80 respondents who depended on plant recourses for managing livestock aliments and showed interest to participate in the study. From the total respondents, 32 were selected purposely by the recommendation of the local elders due to their long practice in providing services related to traditional herbal medicines. Whereas, 48 were selected purposely by the researchers.

Data collection

Ethnobotanical data collections were made through semi-structured questionnaire supported by field observation. The questionnaire was written in English version that was translated to local language (Oromiffa*)*. Group discussions were made with key informants for cross-checking and verifying the information that have been gathered from individuals, and information which joined common understandings considered as relevant otherwise discarded. The collected specimens of medicinal plants were coded with vernacular names, pressed, dried and taken to botanical identification by botany specialists at national Herbarium of Addis Ababa University, College of Natural Science, Department of Plant Biology and Biodiversity. While collecting the medicinal plants, local name, general description of the plants, plant parts used, methods of preparation, routes of administration, aliments to be treated, and demographics parameters of respondents and source of knowledge were properly documented.

Data analysis and management

Collected ethno-botanical data were entered into Microsoft Excel spread sheet and analyzed using SPSS 20 software version and summarized using descriptive statistical methods such as frequency and percentages.

Fidelity value

Fidelity Level (FL), the percentage of informants claiming on the use of a certain plant for the same major purpose was calculated for the most frequently reported diseases or ailments as described by Alexiades (1996).

FL=Ip/Iu X 100
Where
FL =Fidelity Level
Ip = the number of informants that claim a use of a plant species to treat a particular disease
Iu =the number of informants that use the plants as a medicine to treat any given disease

Preference ranking

Preference ranking was also computed using fifteen key informants to assess the most effective medicinal plant species used for treating the most prevalent livestock aliments reported in the area. For this purpose, key informants were selected and asked to assign use values (5=best, 4= very good, 3=good, 2 =less used and 1=least used) to each species. The values given to each medicinal plant were summed and ranked according to Martin (1995) and Cotton (1996).

Results

Demographics of the respondents

A total of 80 respondents were interviewed based on their willingness to share their indigenous knowledge on ethno-veterinary medicine. Of these respondents, the majority of them were male, 63(78.8%) and the remained, 17(21.25%) were females. The age of the respondents was ranged from 17 to 80 years, but their knowledge on medicinal plants was varied. In comparison of educational status of the respondents, non-educated respondents handled much knowledge of traditional medicine than educated one (Table 1).

Inheriting knowledge of medicinal plants

The result of this study disclosed that most of the informants 68.75 % (55/80) have indigenous knowledge on herbal medicine, whereas the rest 31.25 % (25/80) have no any indigenous knowledge on ethno-veterinary medicine. Accordingly, majority of the respondents (90.91%) acquired the knowledge from their parents, 5.46% got by asking local herbalists and the rest (3.64%) by trial and error on their own animals. Transfer of ethnoveterinary knowledge mostly follows vertical transfer to the most selected family member orally with great secrecy (Table 1).

Table 1. Demography of the respondents and sources of endogenous knowledge on ethno-veterinary medicine in the study area.

Parameters	Respondents	Have Ethno-veterinary Knowledge	Percent (%)
Sex			
Male	63	48	76.2
Female	17	17	41.2
Age			
<20	6	1	16.7
20-40	31	15	48.4
>40	43	39	90.7
Educational level			
Illiterate	34	30	88.2
Elementary	25	19	76.0
High school	17	6	35.4
Diploma &above	4	-	-
Inheriting knowledge			
From Parents	55	50	90.9
Asking local herbalists	55	3	5.5
Through trial error	55	2	3.6

Identified Medicinal Plants for Treatment of Livestock Ailments in Wolmera district

This study documented 25 medicinal plant species in 22 families as useful in traditionally managing fifteen different livestock aliments in Wolmera district. Some species were recorded as being used for more than one purpose. Among the recorded species, *Selanecio gigas (Votke) C. Jeffrey*, *Allium sativum L.*,*Rumex nepalensis* Spreng, *Capsicum annuum L.* and *Ferula communis L.* were the most popular plants used to make traditional medicines in the treatment of more than one livestock aliments. Further investigation on the families has shown that families Asteraceae, Euphorbiacea, Solonaceae and Polygonaceae were represented by 2 species each. The remaining (18 families) were represented by single plant species (Table 2).

Medicinal Plant Parts Used, Method of Preparation and Routes of Administration to Treat Livestock Ailments

As shown in table 3, herbalist in Wolmera district used leaf, fruits, stems,

seeds and roots of medicinal plants to treat different animal aliments; and leaf (68%) was found to be the most familiar plant part for remedy preparations to treat blackleg, anthrax, bloat and ecto and endo-parasites. Different methods of ethnoveterinary medicinal plant preparations were also reported from identified plant parts. However, concoction (60%) followed by powdering (28%) were found to be the most frequently used methods of preparation (Table 3).

The herbal drugs following preparation were reported to be administered through different routes. The routes and method of applications varies with the type of disease to be treated and the actual sites of the disease. However, the dominant route of administration was oral (80%) followed by topical (16%) and nasal (4%) (Table 3). The dose regime was generally dependent on the degree and duration of the ailment, age, size and body condition of the animals. Various house hold utensils were used to determine the dose of the local plant medicines (Figure 2).

Table 2. Medicinal plants having ethno-veterinary importance in Wolmera district.

Scientific name of the Plants	Family name	Local name (Oromiffa)	Aliments that can be treated	Plant Parts used	Formulation	Administration route
Phytolacca dodecandra L' Herit	Phytolaccaceae	Andode	Eye disease	Leaf	Squeezing	Topical
Croton macrostachyus Del	Euphorbiaceae	Makanisa	Black leg skin wound	Leaf	Leaves are pounded and squeezed with water	Topical Nasal
Kalanchoe petitiana A. Rich	Crassulaceae	Bosoqe	Internal parasite	Leaf	Chopping, add to hot water, filtered	Per os
Lagenaria cineraria (Molina) standley (LS)	Cucurbitaceae	Buqqe	Black leg Retained placenta	Fruit	powdering and mixed with water, filtered	Per os
Calpurnia aurea (Aiton) Benth.	Fabaceae	Ceka	Rabies, External parasite	Leaf and stem	Fresh leaves are made to paste by adding little water and rubbed the Skin. Powderr them separately then, add to hot water to make a concoction. (rabies)	Topical Per os
Ocimum lamiifolium Hochst	Lamiaceae	Damakase	Black leg, Diarrhoea	Leaf	Grinding, mix with hot water	Per os
Justicia schimperiana (Hochst.exNees) T. Anders	Acanthaceae	Dhumuga	Rabies, black leg	Leaf and root	Powder them separately then, add to hot water to make a concoction.	Per os

Scientific name of the Plants	Family name	Local name (Oromiffa)	Aliments that can be treated	Plant Parts used	Formulation	Administration route
Vernonia amygdalina Del.	Asteraceae	Ebicha	Internal parasite, Retention placenta	Leaf	Chopping, mix with water, filtered	Per os
Solanecio gigas (Vatke.) C. Jeffrey	Asteraceae	Gomanaosolee	Diarrhea, Black leg Colic, Wound	Leaf	Chopping, mix with water	Per os Topical
Verbascum sinaiticumBenth.	Scrophulariaceae	Gurahare	Anthrax	Leaf	Squeeze and drenched the juice	Per os
Ajuga integrifolia Buch	Lamiaceae	Harmagusa	Diarrhea	Leaf and root	Chopped together mix with water	Per os
Ferula communis L.	Apiaceae	Hinsilale	Colic, Bloat Diarrhea	Leaf	Chooped mixed with water	Per os
Allium sativum L	Amaryllidaceae	Kulubiadi	Coughing, fungal infection, Leech	Stem	Chopping, mixing with water	Per os Topical
Rumex nepalensis Spreng.	Polygonaceae	Shulti	Internal parasite External parasite, Wound	Root	Chopping and mix with water	Per os /Topical
Nicotiana tabacum L.	Solanaceae	Tambo	Snake poisoning Leech	Leaf	Powdered leaf	Topical
Ruta chalepensis L.	Rutaceae	Tenadami	Bloat	Leaf	Boiled in hot water, filtered	Per os
Capsicum annuum L	Solanaceae	Barbare	Colic, bloat, Internal parasites	Seed	Grinding and mix with water	Per os
Juniperus procera HochstexEndl.	Cupressaceae	Gatirahabasha	Colic	Leaf	Leaves are pulverized	Per os

Scientific name of the Plants	**Family name**	**Local name (Oromiffa)**	**Aliments that can be treated**	**Plant Parts used**	**Formulation**	**Administration route**
Hagenia abyssinica(Bruce) J. F. Gmel.	Rosaceae	heto	Internal parasites	fruit	Grinding and mixing with water	Per os
Ricinus communis L.	.Euphorbiaceae	Kobo	FMD	Leaf	Chopping and mixing with water	Topical
Cymbopogon citratus	Poaceae	Xajisara	Bloat	Leaf	Chopping and mix with water and filtered	Per os
Clematis simensisFresen.	Ranunculaceae	Hidafiti	Rabies	Root	Chopping, mixing with water and filtered	Per os
Zingier officinale Roscoe	Zingiberaceae	Jinjibila	Eye inflammation	Stem	Crushing	Topical
Rumex abyssinicus Jacq.	Polygonaceae	Meqmeqo	External parasite	Leaf	Drying, powdered	Topical
Stephania abyssinica (Dillon ex A. Rich.) Walp.	Menispermaceae	Kalala	Rabies	Leaf	Chopping, mixing with water and filtered	Per os

Table 3. Summary of plant part(s) used, formulation and routes of administration of medicinal plant in the study area.

Parameters	Frequency	Percent (%) (n=25)
Plant parts used		
Leaf	17	68
Stem	4	16
Root	2	8
Seed	1	4
Fruit	1	4
Formulation		
Concoction	15	60
Squeezing	3	12
Crushing	7	28
Routes of administration		
Oral	20	80
Topical	4	16
Nasal	1	4



Figure 2. Different house hold utensils used to measure plant remedies:
A=Ladle, B= Spoon, C= water glass, D= Plastic Water container, E= horn cup, F= local beer utensil

Relative healing potential of medicinal plants

Capsicum annuum showed highest FL value (82.2%) for the treatment of bloat and under the gastrointestinal therapeutic category, highest fidelity level value was recorded for *Ocimum lamiifolium Hochst* (79.3%). *Calpurnia aurea (Aiton) Benth* was also showed high healing potential against external parasites (75%) as shown in Table 4.

Preference Ranking of Ethno-veterinary Plants

Preference ranking of five medicinal plants that were reported as an effective treatment for internal parasite, which is the most common disease in the study area was conducted after selecting 15 key informants. Hagenia abyssinica (Bruce) J. F. Gmel (64%), followed by Vernonia amygdalina Del (61%) was the most preferred plant species for the treatment of internal parasite in the study district (Table5).

Table 4. Fidelity level value of medicinal plants commonly reported against a given veterinary ailment category.

Medicinal plant species	Therapeutic category	Ip	Iu	FL value (%)
Croton macrostachyus Del	Wound	23	35	65.7
Capsicum annuum L	bloat	23	28	82.2
Rumex nepalensis Spreng	Internal parasite	13	30	43.3
Lagenariasiceraria(Molina) standley (LS)	Retained placenta	20	31	64.5
Nicotiana tabacum L.	Leech	26	38	68.4
Allium sativum L	Respiratory disease	12	28	42.8
Ocimumlamiifolium Hochst	Diarrhea	23	29	79.3
Calpurnia aurea (Aiton) Benth	External parasite	18	24	75

Ip = the number of informants that claim a use of a plant species to treat a particular disease, Iu =the number of informants that use the plants as a medicine to treat any given disease, FL= Fidelity Level

Table 5. Preference ranking of medicinal plants reported for treating internal parasite in livestock.

Medicinal plants for Internal parasite	Respondents laved from R_1 up to R_{15}																
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	R_{13}	R_{14}	R_{15}	**Total score**	**Rank**
Hagenia abyssinica (Bruce) J. F. Gmel.	4	5	4	4	3	5	4	4	5	5	5	4	3	4	5	64	1st
Capsicum annuum L	3	2	3	5	4	2	2	5	2	3	2	1	5	1	3	43	3rd
Rumex nepalensis Spreng	2	3	2	2	5	3	1	4	4	1	3	3	3	3	2	41	4th
Vernonia amygdalina Del.	5	4	5	3	4	4	5	2	3	4	4	5	4	5	4	61	2nd
Kalanchoe petitiana A. Rich	1	1	1	1	1	2	3	3	1	2	1	2	1	2	1	23	6th

NB-Scores in the table indicate ranks given to medicinal plants based on their efficacy. Highest number (5) for the medicinal plant which informants' thought was most effective in treating internal parasites and the lowest number (1) for the least-effective plant species
R1....R15= respondents.

Discussion

The present study was conducted in Wolmera district of Oromia region, Ethiopia from November 2016 to March 2017 to document indigenous knowledge of the communities on Ethno-veterinary medicine. A total of 80 respondents were interviewed based on their willingness to participate in the survey. Of these respondents, only 68.75% had ethno-veterinary knowledge; and most of them were males. The result is in line with studies conducted previously in different parts of Ethiopia such as in Borena pastoralist area (Sori *et al*., 2004), in Jimma zone (Yigezu *et al*., 2014), in Ankober,North Shoa zone (Lulekal *et al.,*2014) and elsewhere in creator Giyai municipality, South Africa (Luseba and Van Der Merwe, 2006) and in North western Yunnan, China (Shen *et al*., 2010). This similarity may be due to high degree of secrecy on passing indigenous knowledge on medicinal plants within the family circle only to sons; and could also be related with the local attitude of societies that restricts female to stay home just only for limited activities such as to look after babies and giving attention to the work performed in the home rather than reacting with outside environment.

In line with this study, other studies have reported that the highest medicinal plant knowledge is developed as the age of the herbalists increased (Yirga *et al*., 2012; Tamiru *et al*., 2013). This may be due to the fact that indigenous knowledge is built with years of experience and might also be the herbalist passed the secret to selected members of the family especially to their elder son when his age become above 40 years.

In this study, 25 species of medicinal plants belong to 22 families were identified and documented for treating 15 types of livestock aliments in study area. Studies conducted previously in different parts of Ethiopia identified and documented different plant species having value in Ethno-veterinary Medicine. *Viz*, 83 plant species in Southern Tigray (Giday and Ameni,2003), 43 species in Borona pastoralist area (Sori *et al*., 2004), 72 species in Wonago district, Southern Ethiopia (Mesfin *et al*.,2009), 22 species in Seharti-Samre district (Yirga *et al*., 2012),74 species in Jimma Zone (Yigezu *et al*., 2014),51 species in Ankober, North Shoa (Lulekal *et al*.,2014) and 24 species in Amaro special district (Tekele, 2015). These identified and documented medicinal plant species were used for treating fifteen to thirty-seven livestock aliments in each study sites. Overall, our survey and works done previously in different parts

of Ethiopia have shown that the country is rich in medicinal plants and fairly distributed throughout its regions.

Among medicinal plant species reported in this study, 9 species in Horogudru districts (Tadesse and Dereje, 2015), 6 species in South Ethiopia (Romha *et al*.,2015), 2 in central Kenya (Njoroge and Bussmann, 2006), 3 in Marajó Island, Brazil (Monteiro *et al*.,2011) and 3 in central India (Verma, 2014) were reported to treat almost similar livestock aliments. All these findings suggest that traditional ethno-veterinary knowledge widely used in elsewhere in the world and there might be increases inclination of people to depend on such practices because it is considered equivalently effective to modern cares, and moreover, cheap and easily available in their environments (Yigezu *et al*.,2014).

Some of the medicinal plants reported were also described in other surveys to treat similar diseases. *Vernonia amygdalina Del*. And *Agugain tergrifolia* F.Gmel(Tekele, 2015) were used to treat internal parasites. Also, *Capsium annuum L* and *Zingiber officinale* Roscoe(Lulekal *et al*., 2014; Tadesse and Derje, 2015) were used to treat bloat in Cattle, *Zingiber officinale Roscoe* (Yigezu *et al*.,2014) for conjunctivitis, *Justicica schimperiana (Hochst.exNees)* T.anders (Tadesse and Derje, 2015) for rabies, *Nicotiana tabacum L. (*Yigezu *et al*., 2014; Romha *et al.,*2015; Takele, 2015) was used to treat snake bite and leech and *Croton macrostachyus* Del. and *Calpurnia aurea* (Aiton) Benth (Lulekal *et al*., 2014) also reported to treat wound and tick infestation respectively. Medicinal plants that were reported by different informants and communities with the same therapeutic indications represent traces of true efficacy (Giday *et al*., 2009).

Alike present study, leafy part of the plants rank first, among the various plant parts(stems rots and seeds) used for treatment of various diseases of the livestock in other studies at different parts of Ethiopia (Tamiru *et al*., 2013; Yigezu *et al*., 2014; Romha *et al*., 2015; Tekele, 2015), in India (Verma, 2014), in Brazil (Monteiro *et al*.,2011), in Pakistan (Deeba *et al.,* 2009) and in Kenya (Njoroge and Bussmann, 2006); however, in contrast to our study, Lulekal *et al*., (2015), Mesfin *et al*.(2009) and Tabuti *et al*. (2003) have reported that root is as the most used part in their studies. This difference could be as the pharmaceutical value and concentration of active ingredients in each plant varied depending on climatic and geographical factors, soil type, and difference in endogenous knowledge in the communities; and might be also the types of medicinal plant species found in the study areas. However, harvesting of leaves compared to

harvesting of stems and roots has a less negative impact on the survival and sustainable utilization of useful medicinal plants in future perspective (Yigezu *et al*., 2014*).*

Herbalists in the study area were used a variety way to prepare their plant remedies such as concoction, crushing/powdering and squeezing and administered the prepared remedies per os or applied topically. These findings were concurred with the reports of Yigezu *et al*. (2014) in Jimma zone, Mesfin *et al*. (2009) in Wonago, Ethiopia, Deeba *et al*. (2009) in Pakistan, Njoroge and Bussmann (2006) in Kenya and Shen *et al*. (2010) in Nu Village of western China. These methods of preparation and routes of administration might be related to usage of different solvents mainly water as vehicle system; and could be also considered as rapid physiological reaction with the causative agents and increase the curative power of the medicinal plant remedies.

Determination of dose of plant medicine was determined by severity and duration of ailments, body size and age of the patients. However, there were variations in unit of measurement of plant remedies. Most of the time, doses in the study area were determined by using different house hold utensils like water glasses, ladles, horn cups, spoons and plastic containers. On the other hand, lack of precision and standardization has been reported as a drawback of remedy preparation from medicinal plants. Similar findings were reported in Ethiopia and elsewhere in the world (Yineger *et al*., 2008; Deeba *et al*., 2009; Monteiro *et al*., 2011; Lulekal *et al*., 2014; Romha *et al*., 2015).

Healing potential for some medicinal plants was estimated using Fidelity level (FL). Accordingly, *Capsicum annuum L.* (82.2%), followed by *Calpurnia aurea (Aiton*) (75%) were the plants having the highest FL value, for their use to treat bloat and external parasites, respectively. Similar findings have been reported in other studies (Lulekal *et al*., 2014; Romha *et al*., 2015). Plants scoring higher FL values are thought to have better potency as compared to plants with less FL values (Trotter and Logan 1986). On the other hand, Lulekal *et al*. (2013) recommended for further chemical screening those plants with high FL values.

Hagenia abyssinica (Bruce) J. F. Gmel and *Vernonia amygdalina Del.* were the most preferred medicinal plant species to treat internal parasites, the most prevalent disease in the area. This might be attributed to the presence of bioactive compounds against different internal parasitic species

Conclusion and Recommendation

Ethno-veterinary practices significantly play a major role in livestock health care as an alternative or integral part of modern veterinary practices especially in marginal areas where modern veterinary coverage is lacking. In this survey, about 25 medicinal plant species belong to 22 families were identified and documented. The people in the study district have ample ethno- veterinary knowledge in treatment of livestock aliments of different origins. However, the efficacy and the toxicity level of each medicinal plant species were not well known. Therefore, we strongly recommended for detail scientific and pharmacological evaluations (efficacy, safety, mode of delivery and dosage) of the identified plant species in future use.

Acknowledgments

We are thankful to the people of the Wolmera district, Oromia Region for their participation in this study and for sharing their valuable knowledge and experience. We are also grateful to national Herbarium of Addis Ababa University staffs for their valuable help in plant identification.

Conflict of interest

There is no conflict of interest to declare

References

Alexiades, M.,1996. Collecting ethnobotanical data: An introduction to basic concepts and techniques. Selected Guideline for Ethnobotanical Research: A Field Manual. Edited by: Alexiades M, Sheldon JW., Bronx, New York: The New York Botanical Garden.

Briggs, P., 2002. Ethiopia, The Bradt Travel Guide, 3rd edition, Chalfont St Peters: Bradt, p. 481.

CSA (Central Statistical Agency). 2015. Agricultural sample survey of 2014/2015, report on livestock and livestock characteristics, Addis Ababa, statistical bulletin, V II, p. 40.

Cotton, C.M., 1996. Ethno-botany: Principles and Applications. John Wiley and Sons Ltd. Chichester, England.

Deeba, F., Muhammad, G., Iqbal, Z. and Hussain, I., 2009. Survey of ethno-veterinary practices used for different ailments in dairy animals in peri-urban areas of Faisalabad (Pakistan). *Int. J. Agro. Biol.,*11(5)*,* 535-541.

Endashaw, B.,2007. Study on actual situation of medicinal plants in Ethiopia, Prepared for JAICAF (Japan Association for International Collaboration of Agriculture and Forestry) pp.1-66.

Giday, M. and Teklehaymanot, T., 2013. Ethnobotanical study of plants used in management of livestock health problems by Afar people of Ada'ar district, Afar regional state, Ethiopia. *J. Ethnobiol. Ethnomed.,*9,1-8

Giday, M., and Ameni, G., 2003. An ethnobotanical survey on plants of veterinary importance in two districts of Southern Tigray, Northern Ethiopia. *Ethiop. J. Sci.*, 26 (3), 123-136.

Giday, M., Asfaw, Z. and Woldu, Z., 2009. Medicinal plants of the Meinit ethnic group of Ethiopia. An ethnobotanical study. *J. Ethnopharmacol.*,124(3), 513-521.

Hunde, D., Asfaw, Z., Kelbessa, E. 2004. Use and management of ethnoveterinary medicinal plants by indigenous people in 'Boosat', Welenchetti area, Ethiopia. *Ethiop. J. Biolo. Sci.*, 3**,**113-132.

Kalayou, S., Haileselassie, M., Gebre-egziabher, G., Tikue, T., Sahle, S., Taddele, H., and Ghezu, M., 2012. *In-vitro* antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. *Asian Pac. J. Trop. Biomed.,*2, 516-522.

Kaur, R., Kapoor, K. and Kaur, H., 2011. Plants as a source of anticancer agents. *J. Nat. Prod. Plant Resour.,*1, 119–24.

Kebu, B., Ensermu, K.and Zemede, A., 2004. Indigenous medicinal utilization, management and threats in Fentale area, Eastern Shewa, Ethiopia. *Ethiop. J. Biolo. Sci.*, 3(1), 37-58.

Khan, M. and Hussain. M., 2012. Ethno-Veterinary Medicinal Uses of Plants of Poonch Valley Azad Kashmir. *Pak. J. Weed. Sci. Res.,* 18,495-507.

Lulekal, E., Asfaw. Z., Kelbessa. E., and Van Damme. P., 2014. Ethnoveterinary plants of Ankober District, North Shewa Zone, Amhara Region, Ethiopia. *J. Ethnobiol. Ethnomed.,* 10(21)*,* 2.

Luseba, D., Van Der Merwe, D., (2006). Ethnoveterinary medicine practices among Tsonga speaking people of South Africa. *Onderstepoort J. Vet. Res.*, 73, 115–122.

Martin, J., 996. Ethnobotany, A 'people and Plants' Conservation Manual, Chapman and Hall, pp.268.

Mesfin, F., Demissew, S., Teklehaymanot, T., 2009. An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *J. Ethnobiol.Ethnomed.,* 5(28) .https://doi.org/10.1186/1746-4269-5-28.

Monteiro, M. V., Bevilaqua, C. M., Palha, M., Braga, R. R., Schwanke, K., Rodrigues, S. T.and Lameira, O.A., 2011. Ethnoveterinary knowledge of the inhabitants of Marajó Island, Eastern Amazonia, Brazil. *Acta Amaz.,* 41(2), 36-47.

Njoroge, G. N.and Bussmann, R. W., 2006. Herbal usage and informant consensus in ethnoveterinary management of cattle diseases among the Kikuyus (Central Kenya). *J. Ethnopharmacol.,* 108(3). 332-339. doi:10.1016/j.jep.2006.05.031.

Romha, G., Dejene, T., Telila, L.and Bekele, D. 2015. Ethnoveterinary medicinal plants: Preparation and application methods by traditional healers in selected districts of southern Ethiopia. *Vet. World.,* 8 (5), 674-684.

Shen, S., Qian. J., Ren, J., 2010. Ethnoveterinary plant remedies used by Nu people in NW Yunnan of China. *J. Ethnobiol. Ethnomed.*, 6 (24), 1186-1124.

Somvanshi, R., 2006. Veterinary medicine and animal keeping in ancient India. *Asian Agri-his*., 10(1),133-146.

Sori, T, Bekana, M., Adugna, G., .and Kelbessa, E., 2004. Medicinal plants in the ethnoveterinary practices of Borana Pastoralists, Southern Ethiopia. *Int. J. Appl. Res. Vet. Med.*, 3(2), 220-225.

Tabuti, J. R, DhillionS. S. and Lye, K.A., 2003. Ethno-veterinary medicines for cattle (*Bos indicus*) in Bulamogi county Uganda: plant species and mode of use. *J. Ethnopharmacol.*, 88,279-286.

Tadesse, B., and Dereje, A., 2015. Survey of ethno-veterinary medicinal plants at selected Horro Gudurru Districts, Western Ethiopia. *Afr. J. plant sci.*, 9(3),185-192.

Tafara, M., and Taona, B., 2004. Ethno-veterinary medicine: A potential alternative to orthodox animal health delivery in Zimbabwe. *Int. J. Vet. Med.*, 2 (4),269-273.

Tekele, Y. 2015. Study on Ethno-veterinary Practices in Amaro Special District Southern Ethiopia. *Med. Aromat. Plants*, 4(2), 1-8.

Tamiru, F., Waktole, T., Ejigu, K., Gizaw, D., Kumar. R. and Sorsa, M., 2013. Ethnoknowledge of plants used in veterinary practices in Dabo Hana District, West Ethiopia. *J. Med. Plants Res*., 7(40), 2960-2971.

Trotter, R. *T. and Logan, M. H., 1986. Informants consensus: a new approach for identifying potentially effective medicinal plants. Plants in Indigenous Medicine and Diet. Edited by: Etkin NL, Bedford H., New York: Redgrave Publishing Company.*

Vander, M., Swan, G., Botha, C.J.,2001. Ethno-veterinary medicinal plants in Northwest province of South Africa. *J. S. Afr. Vet. Assoc.*, 72(4),189-196.

Verma, R.K., 2014. An ethnobotanical study of plants used for the treatment of livestock diseases in Tikamgarh District of Bundelkhand Central India. *Asian Pac. J. Trop. Biomed*., 4 (Suppl 1): S460-7. doi:10.12980/APJTB.4.2014C1067.

Yigezu. Y., Berihun., D.and Yenet. W., 2014. Ethnoveterinary medicines in four districts of Jimma zone, Ethiopia: cross sectional survey for plant species and mode of use. *BMC Vet. Res*., 10(76). doi.org/10.1186/1746-6148-10-76.

Yinegar, H., Kelbessa, E., Bekele, T. and Lulekal, E., 2007. Ethnoveterinary medicinal plants in Bale Mountains National Park, Ethiopia. *J. Ethnopharmacol.*,112 (1), 55-70.

Yineger, H., Yewhalaw, D. and Teketay, D., 2008. Plants of veterinary importance in Southwestern Ethiopia, The case of Gilgel Ghibe area. *Forest Trees Livelihoods*, 18 (2),165-181.

Yirga, G., Teferi, M., Brhane, G., .and Amare, S., 2012. Plants used in ethnoveterinary practices in Medebay-Zana District, Northern Ethiopia. *J. Med. Plants Res.,*6(3),433-438.

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_____