

OPEN ACCESS



Journal of  
**Veterinary Medicine and Animal Health**

June 2018  
ISSN 2141-2529  
DOI: 10.5897/JVMAH  
[www.academicjournals.org](http://www.academicjournals.org)

 **ACADEMIC  
JOURNALS**  
expand your knowledge

## ABOUT JVMAH

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

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

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

### Contact Us

Editorial Office: [jvmah@academicjournals.org](mailto:jvmah@academicjournals.org)

Help Desk: [helpdesk@academicjournals.org](mailto:helpdesk@academicjournals.org)

Website: <http://www.academicjournals.org/journal/JVMAH>

Submit manuscript online <http://ms.academicjournals.me/>.

## **Editors**

**Dr. Lachhman Das Singla**

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

**Dr. Viktor Jurkovich**

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

## Editorial Board Members

**Dr. Adeolu Alex Adedapo**

*Department of Veterinary Physiology  
Biochemistry and Pharmacology  
University of Ibadan  
Nigeria*

**Prof. Anca Mihaly Cozmuta**

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

**Dr. Ramasamy Harikrishnan**

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

**Dr. Manoj Brahmbhatt**

*Department Of Veterinary Public Health & Epidemiology,  
College Of Veterinary Science,  
Anand Agricultural University,  
Anand,  
India*

# Journal of Veterinary Medicine and Animal Health

Table of Contents: Volume 10 Number 6 June 2018

## ARTICLES

- Mechanism of retained placenta and its treatment by plant medicine in ruminant animals in Oromia, Ethiopia** 135  
Tagesu Abdisa
- A study on gross and histopathological pulmonary lesions of cattle slaughtered at Abergelle Abattoir, Mekelle, Tigray, Ethiopia** 148  
Kidane Workelul Yalew, Nesibu Awol, Yishak Tsegay, Haftay Abraha and Hailesilassie W/mariam
- Study on calf coccidiosis in dairy farms in and around Holeta Town, Finfine Zuria Liyu Zone, Oromia, Ethiopia** 153  
Zerihun Adugna Regasa, Belay Mulatea and Temesgen Kassa Getahun
- Determination of pH and water holding capacity of beef from selected butcher shops of Mekelle, Ethiopia** 159  
Haileslassie W/mariam Gebrehiwot, Endale Balcha, Yohanes Hagos and Kidane W/rkelul

*Review*

# Mechanism of retained placenta and its treatment by plant medicine in ruminant animals in Oromia, Ethiopia

**Tagesu Abdisa**

School of Veterinary Medicine, Jimma University, Jimma, Oromia, Ethiopia.

Received 1 February, 2018; Accepted 18 April, 2018

Retained placenta is the abnormal condition in animal reproduction in which fetal membrane failed to detach from maternal uterine wall after postpartum. The retention of placenta can create some disorders within reproductive organ of animals by allowing microorganisms to grow inside the uterus causing inflammation of uterus, fever, weight loss, decreasing milk yield, longer calving intervals and if the case is severe animals may die. The pathogenesis pathway of retained placenta development commences with an imbalance of antioxidant or oxidant capacity, decrease in estrogen production, decreased prostaglandin F2 alpha, and accumulation of arachidonic and linoleic acids in the placental tissue. Retained placenta can be treated with different methods such as manual removal, administration of intrauterine antibiotics, administration of hormonal therapy, and the use of plant medicine. The most common parts of plants used for drugs preparation are the leave, bark, root, stem and seed of the specific plant. The route of drug administration to animal is oral and vagina infusion. The herbal or plant prescriptions for treatment of placenta retention are characterized as antibiosis, antiphlogosis, immune enhancement and fertility improvement without endometrial injuries. The plants which have been used as treatment of retained placenta in Ethiopia are Flax seed, *Vernonia amygdalia*, *Dodonea angustifolia*, *Solanum acaule*, *Solanum acuminatum*, *Dovyalis* spp., *Galinsoga quadriradiata*, *Plumbago zeylanica*, *Momordica* spp., *Colocasia esculenta*, *Bryophyllum pinnatu* and *Urera hypselodendron*. The main objective of this paper is to illustrate the mechanism of retained placenta and its treatment using plant medicine in current status which helps the pastoral and clinician at remote areas and also to give enough information on the plant species that are sources of different chemical ingredient for future production of modern drug treatment for retained placenta.

**Key words:** Antibiosis, antiphlogosis, ethinoveterinarian, retained of placenta, treatment.

## INTRODUCTION

Reproductive disorders are one of the major causes of decrease in reproductive efficiency and determinant of lifetime productivity of cows. The major causes of reproductive disorder in animals are abortion, dystocia,

retained fetal membrane, pyometra, metritis, uterine and vaginal prolapsed, anoestrus and repeated breeding (Tagesu, 2018). Retention of placenta (ROP) is the abnormal condition in which fetal membrane failed to

\*Corresponding author. E-mail: [abdusatagesu@gmail.com](mailto:abdusatagesu@gmail.com), [atagish5@gmail.com](mailto:atagish5@gmail.com). Tel: +251933681407.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

detach from maternal uterine wall after postpartum. It is also called retention of fetal membrane (RFM). Retention of placenta (ROP) occurs when fetal membrane fail to detach after postpartum within 8 h. Retention of fetal membranes from 6 to 24 h post parturition is termed retained placenta. It has been published in various journals in form of reviews and researches (Biner et al., 2015; Jemal, 2016; Swiefy, 2003; Tucho and Ahmed, 2017). It is better to illustrate the solution for retained placenta in developing countries. Ethnoveterinary have showed a great role in the treatment of animal diseases in developing country. Therefore, this paper stands to illustrate and coin out the problem, and solution to ROP in ruminant animals.

ROP can create some disorders within reproductive organ of animals by allowing microorganisms to grow inside the uterus thereby causing inflammation of uterus, fever, weight loss, decreasing milk yield, longer calving intervals and if the case is severe animals may die. Moreover, the animals which are in retained placenta can suffer from tetanus caused by tetanus organism which is commonly present in faeces and in the soil, and it requires at least 1 to 3 months as long term therapy. ROP can affect the economy of developing country, by decreasing economic growth rate mainly due to decreased milk yield and calf drop (Amin et al., 2013). Although the actual causes of retained placenta are not clear; the condition usually follows dystocia, maternal hypimmunity, mal and unbalanced nutrition, stress, hereditary predispositions or infections (Hanafi et al., 2011).

## **PHYSIOLOGICAL ATTACHMENT AND DETACHMENT OF FETAL MEMBRANE**

Cattle have cotyledonary placentas, wherein the fetal cotyledons are attached and envelop the maternal caruncles, forming the placentome. This connection is facilitated by villi from the cotyledons, and microvilli interactions at the cotyledon-caruncle interface. Collagen links the interface together at several sites, and the breakdown of this collagen is likely a key factor in placental separation (Eiler and Hopkins, 1993; Eiler and FECTEAU, 2007) (Figure 1).

The normal sequence of events initiating parturition involves fetal cortisol induction of placental enzymes that direct steroid synthesis away from progesterone and toward estrogen. Estrogen stimulates parturition by increasing the expression of the genes associated with myometrial excitability and contraction, and increases uterine sensitivity to oxytocin during the course of pregnancy. The increased estrogen can be up regulating the oxytocin receptors on the myometrium and secretion of prostaglandin F<sub>2</sub> alpha (PGF<sub>2</sub>α). Therefore, oxytocin and prostaglandin produce strong contraction of uterine muscle (Fuchs et al., 2001).

Prostaglandin initiates myometrial contractions and results in lysis of the corpus luteum (CL). Lysis of the CL leads to secretion of relaxin and a further decline in progesterone. Both the secretion of relaxin and the decline of progesterone promote collagenase activity. Relaxin is well known for causing collagen lysis resulting in the softening of the cervix and relaxation of the pelvic ligaments. Thus, relaxin might also promote collagen breakdown at the fetal cotyledon-maternal caruncle interface. Conversely, progesterone promotes myometrial quiescence and suppresses collagenase activity. Thus, the decline in progesterone during the prepartum period could make allowances for the enzymatic activity necessary for placental separation (Fuchs et al., 1999; Maj and Kankofer, 1997). The mechanism of placental separation is detailed in Figure 2.

In the normal situation, serotonin plays a great role in the attachment of placenta. High fetal and placental serotonin during pregnancy could help to maintain placental attachment by promoting placental cell proliferation and inhibiting matrix metalloproteinase (MMP) activity (Fecteau and Eiler, 2001). The maturation of the fetal monamine oxidase enzyme system close to parturition could result in the metabolization and subsequent decrease in serotonin, which in turn could promote placental separation and parturition (Fecteau and Eiler, 2001). In addition to changes in the hormonal environment that favors the enzymatic breakdown of cotyledon-caruncle linkages, activation of the maternal immune response against the fetal membranes can play an important role in the breakdown of the placenta. Increased leukocyte chemotaxis and activity occur in cows with normally expelled placentas, and the cytokine interleukin-8 plays the role of a neutrophil chemoattractant in the cotyledon during parturition (Kimura et al., 2002).

The maternal immunological recognition of fetal MHC Class I proteins expressed by trophoblast cells triggers an inflammatory response that contributes to trophoplacental separation. These molecules, absent in early pregnancy, are expressed by fetal trophoblast cells in the 3rd trimester of pregnancy, and could play a role in initiating an inflammatory response that ultimately dissolves the adhesions between maternal and fetal portions of the placenta (Davies et al., 2000, 2004). Trophoblast secretes interferon-tau (IFN-τ) and both trophoblast and endometrium secrete prostaglandin E and the endometrial glands secrete serpins (uterine milk proteins), all of which inhibits lymphocyte activation to keep on the embryo not rejected by the dam (Hauguel-de Mouzon and Guerre-Millo, 2006). Class I MHC antigens expressed by trophoblasts prevent maternal NK-mediated cytotoxic responses (Entrican and Wheelhouse, 2006). Some researches propose that placental retention after normal parturition is more common when the presence of MHC Class I compatibility occurred between the dam and calves. This MHC Class I compatibility

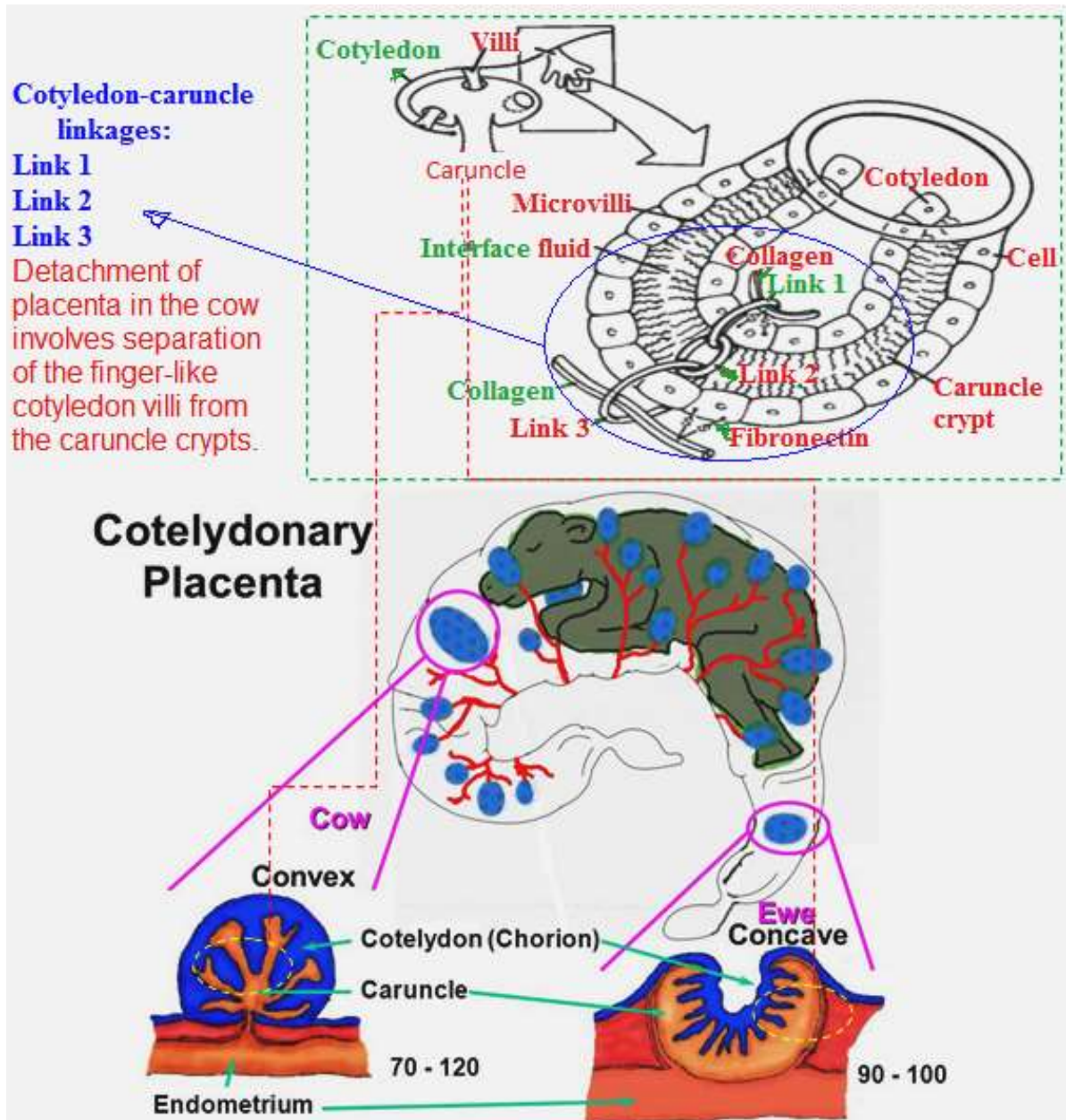


Figure 1. Structure of placenta and cotyledon- caruncle linkage.

implies genetic similarity in the MHC locus between fetus and dam which result in deficient alloreactivity of the maternal immune system against fetal antigens. All this leads to lack of cytokine production such as interleukin-2 and tumor necrotic factor alpha that are necessary for the maturation and shedding of placenta (Davies et al., 2004; Joosten and Hensen, 1992).

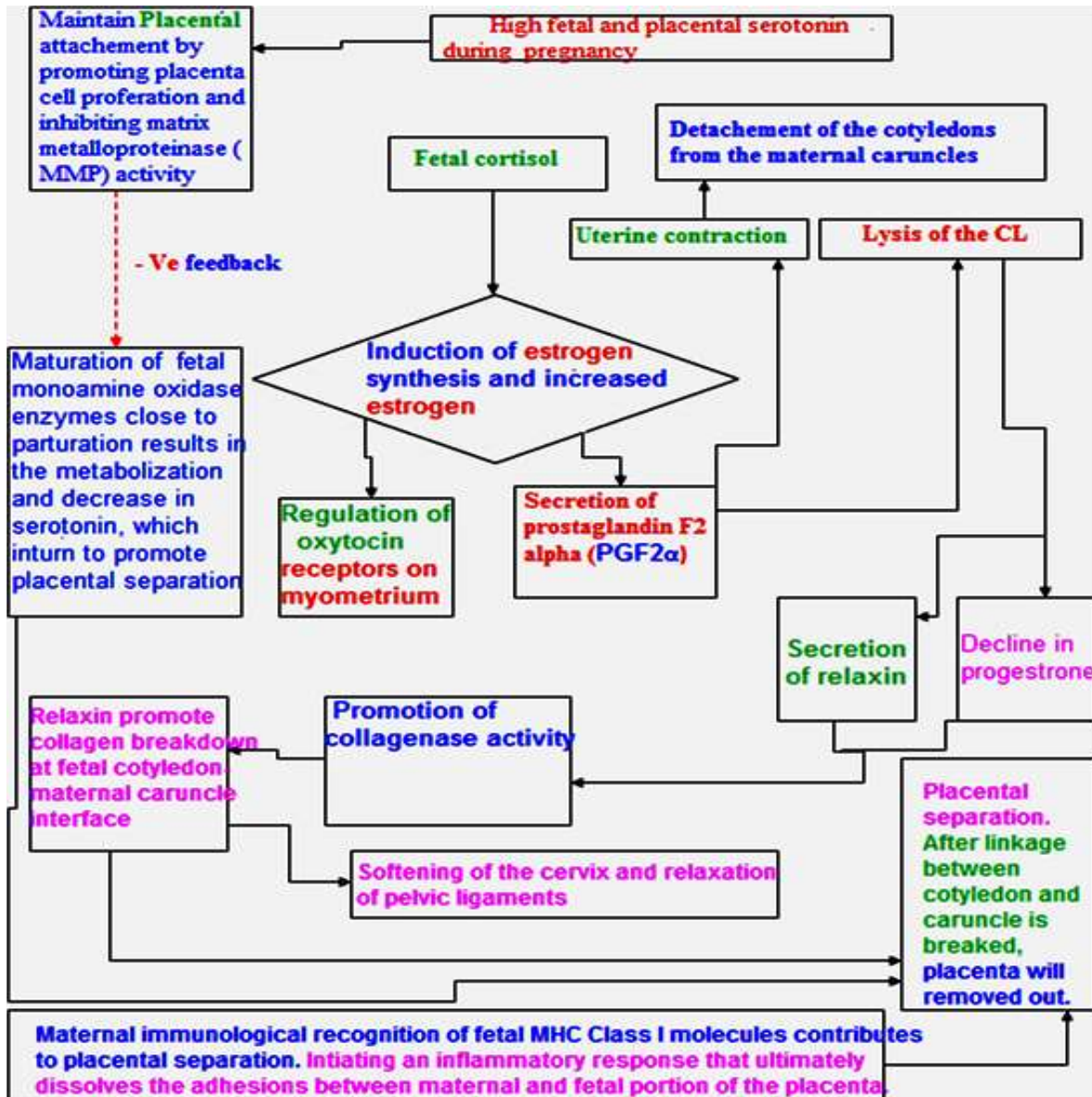
The increased cortisol concentrations in cows that developed retained placenta have immunosuppressive and inhibitory effects on leukocyte migratory activity (Engler et al., 2004). High progesterone and cortisol levels in the blood in stressed cows may induce

accumulation of immunosuppressive proteins in the uterine lumen; this makes the uterus susceptible to infection and persistence of bacteria (Azawi et al., 2008; Kaczmarowski et al., 2006).

#### THE MECHANISM OF PLACENTA RETENTION IN RUMINANT ANIMALS

Pregnancy in dairy cows is considered to induce oxidative stress, which in turn can be a significant underlying factor to dysfunction host immunity and





**Figure 2.** Mechanism of placental separation in domestic animals.

inflammatory responses and can increase the incidence of perinatal disorders (Sordillo and Aitken, 2009). The pathogenesis pathway of retained placenta development commences with an imbalance of antioxidant or oxidant capacity, decrease in estrogen production, decreased  $PGF2\alpha$  and accumulation of arachidonic and linoleic acids in the placental tissue. Therefore, the diminishing effect of antioxidant can cause retained placenta in ruminant animals. It is necessary for the animal organism to maintain its antioxidative/oxidative process in the balance during prenatal period (Sordillo and Aitken, 2009; Wischral et al., 2001).

When the animals approach labor, hormone like prostaglandin and oxytocin are released, resulting in

mechanical contraction of the uterus that is a vital role for normal delivery. The contraction persists into stages of labor and is responsible for expulsion of fetal membrane. However, when the contraction of uterus fails, the fetal membrane stays in uterus without expulsion. Therefore, the main case for retained placenta is the failure of uterine contraction which contributes to the detachment of cotyledons from maternal caruncles, and the lack of damage to fetal villi in normally expelled membrane which is not purely mechanical (Laven and Peters, 1996). Weakness of contraction of myometrium, lesion of cotyledon, edema of villus, heparin released by mast cells and the deficiency of trace minerals are involved in the retained placenta (Hehenberger et al., 2015). In

general, it is suggested that the placenta can be retained in uterus of animal due to lack of uterine contraction and inflammatory disorder which accumulate the arachidonic in placental tissue, also if the collagen which connects the caruncle to cotyledon is unable to breakdown; this causes the persistence attachment of fetal membrane in uterus of animals.

The events involved during detachment of fetal membrane or in cotyledon- caruncle detachment are listed as follows (Youngquist and Threlfall, 2006).

### **Morphological event**

Detachment of placenta in the cow involves separation of the finger-like cotyledon villi from the caruncle crypts without significant tearing of either fetal or maternal epithelia. For the cotyledon villi to separate from the caruncle crypt, it is critical that the mouth of the cotyledon "pouch" be opened first by proteolytic enzymes. The separation of cotyledon from caruncle is achieved when the formation of the depression of the cotyledon toward the apex by digesting the edge of the cotyledon pouch occurred, then the finger-like cotyledon villi will be separated from the caruncle crypt.

After placental detachment is accomplished, uterine involution is completed in an average of 39 days in normal cows and 50 days in cows with retained placenta. By day 6 of postpartum, caruncle septa are disorganized; by day 15, caruncles are completely sloughed as a result of necrosis. Consequently, retained membranes are detached by caruncle necrosis within 6 to 10 days and not later than 17 days of post-partum. The surface of the endometrium is covered with new epithelium by day 26 to 30 post-partum (Youngquist and Threlfall, 2006).

### **Biochemical events**

The biochemistry of uterus after parturition is dominated by increased collagenase and protease activity that correlated with different stages of parturition, resulting in a massive breakdown of collagen and other proteins during uterine involution.

### **Physiological events**

The physiological release of placenta is accomplished in most cows between 3 and 6 h postpartum. Cotyledon proteolysis (dehiscence) and decreasing adhesiveness (viscosity) of the cotyledon-caruncle interface fluids seem to be key factors in the release of placenta. Collagenases are capable of reducing the specific viscosity of collagen. Collagenase activity of cotyledon villi during delivery is increased in healthy cows and decreased in cows with placenta retention (Youngquist and Threlfall, 2006).

The cellular sources of collagenase and proteolytic enzymes for placental release in the cow are unknown. In laboratory animals and humans, myometrial cells, fibroblasts, and leukocytes have been identified as sources of collagenase in the uterus (Youngquist and Threlfall, 2006).

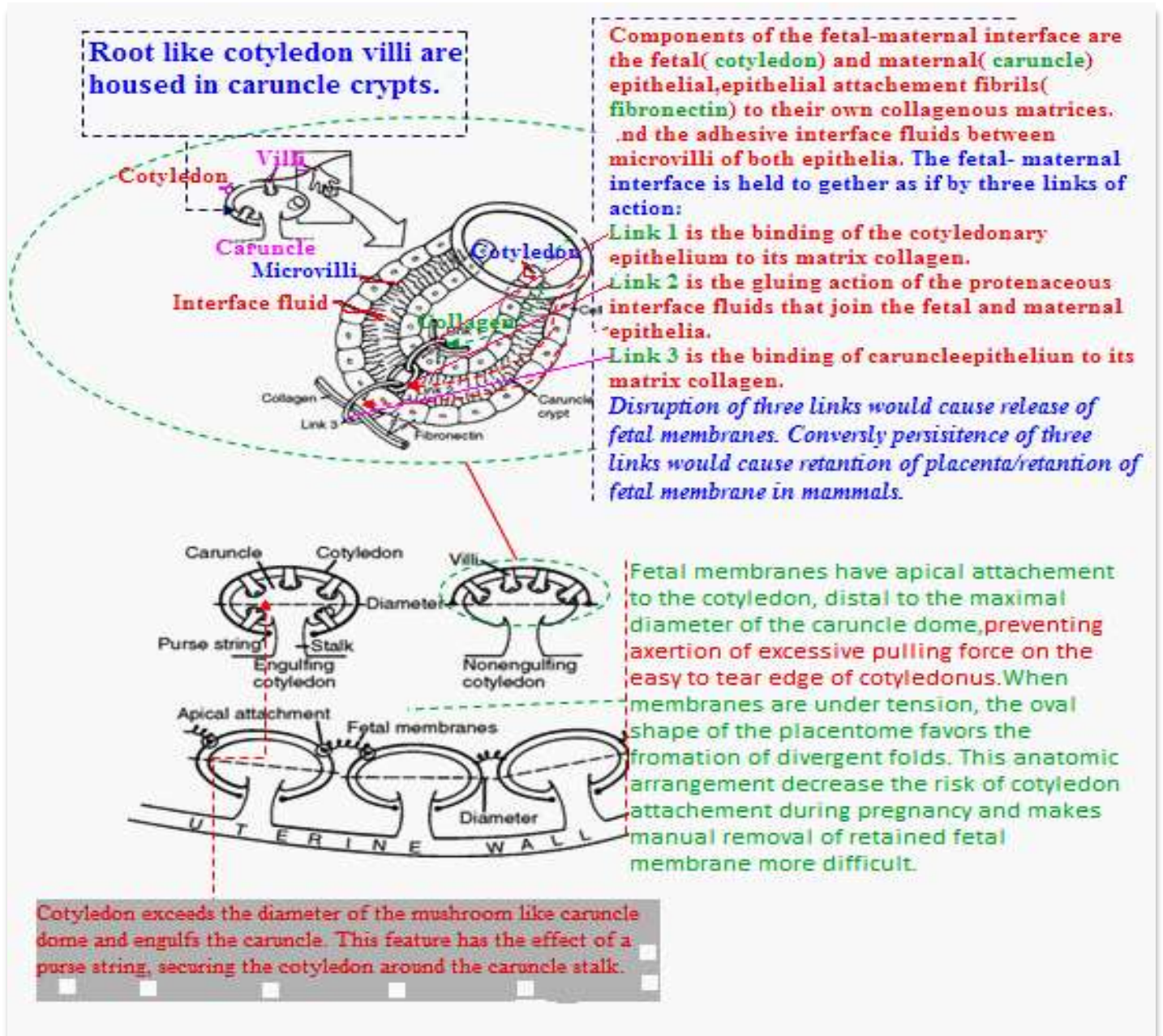
Lack of uterine motility is not considered as a reason for primary retention, since uterine motility is normal or above normal in cows with primary ROP. The direct cause of placental retention is uncertain, but it is related to a deficiency of myometrial contractions and failure of the maternal immune system to successfully degrade the placentomes at the end of pregnancy (Frazer, 2005). The pathogenesis or development of retained placenta results from failure of timely breakdown of the cotyledon-caruncle attachments after delivering of the fetus (Figure 3). The most common causes for ROP are infectious and non-infectious diseases. Abortion, stillbirth, twinning, dystocia, induction of parturition with PGF<sub>2</sub> $\alpha$ , metabolic disorders like milk fevers and caesarian section, can cause retention of placenta (Frazer, 2005).

The spontaneous myometrial contractility is regulated by autocrine and paracrine release of PGF<sub>2</sub> $\alpha$ , the disturbance of endocrine function, high progesterone and cortisol levels, and low oestradiol level traced in the blood of cows with retained placenta (Kaczmarowski et al., 2006). Hormone imbalances existing before delivery are effective in inducing ROP. Increased progesterone level in ROP may be due to failure of the placenta to produce specific steroidal enzymes that help in progesterone aromatization and its conversion to estrogens (Ball and Peters, 2004).

Progesterone, more than estrogen, inhibits uterine collagenases and slows uterine involution. Dexamethasone increases synthesis and utilization of progesterone by cotyledon tissues in the cow. These changes may contribute to blocking postpartum expression of cotyledon collagenases. Moreover, it has been found that glucocorticoids down-regulate collagenases (Youngquist and Threlfall, 2006).

The large granulated binucleate cells in the trophoblast of fetal membrane play a critical role in the parturition process. Binucleate cells produce placental lactogen and pregnancy associated glycoprotein (Ward et al., 2002). The increase in cortisol causes migration of binucleate cells from the fetal side of the placenta to the maternal side. When binucleate cell is migrated and becomes degranulated, there is increase in the concentration of placenta lactogen and other pregnant specific proteins (glycoprotein) (Drillich et al., 2006). These cells fuse with the endometrial epithelium and express MHC Class I on their surface. The villous portion of the placentome is the area of tight attachment and nutrient exchange. MHC Class I expression occurs prior to binucleate cell migration in this areas (Davies et al., 2000).

The cortisol on the fetal side which comes from the



**Figure 3.** The components of caruncle- cotyledon linkage and mechanism of retained placenta.

fetal adrenal gland is the first critical initiation of placental separation or detachment. The fetal adrenal gland is stimulated by adrenocorticotrophic hormone due to maturation of the fetal hypothalamus and stress axis near the time of expected parturition. The fetal cortisol can reach a critical level of about 30 h prior to normal parturition; the increasing cortisol concentration is only on the fetal side of the circulation. Any increase in circulating cortisol in the maternal circulation is due to production of cortisol by the dam adrenal gland (Ward et al., 2002).

## TREATMENT FOR RETAINED PLACENTA IN RUMINANTS

### General fact for treatment of retained placenta in ruminant animals

The treatment of ROP is carried out by detachment of the membrane in order to avoid or reduce the inflammation and other disease, decrease milk losses, and reduce reproductive inefficiency. The therapy treatment of



retained fetal membrane is a controversial subject. Intrauterine antibiotics are routinely administered, although their effect on fertility parameters is under question (Biner et al., 2015). Retained placenta can be treated using different methods such as manual removal, administration of intrauterine antibiotics, administration of hormonal therapy, and the use of plant medicine (Ethnoveterinary methods).

The manual removal of the placenta remains a common practice in every veterinary clinic, despite many studies had failed to demonstrate its benefit on reproductive performance. A study revealed that comparing manual removal and intrauterine antibiotic therapy along systemic treatment of febrile cows found no difference in reproductive outcomes, when compared with the use of systemic therapy of the febrile cows alone (Drillich et al., 2006, 2007).

Manual removal can result in a more frequent and severe uterine infections. More conservative treatment and manual removal prolonged the interval from calving to 1<sup>st</sup> functioning CL by 20 days. Removal of an attached placenta can cause damage to the endometrium and suppresses uterine leukocyte phagocytosis, both of which encourage bacterial invasion and they all have effect on fertility of animals (Drillich et al., 2007; Eiler and Hopkins, 1992). Tetracycline antibiotics commonly used for intrauterine treatment in cattle inhibits matrix metalloproteinase (MMPs) and interferes with the normal placental detachment mechanisms. The breakdown of collagens plays a role in placental detachment and infusion of collagenase which can be helpful in breaking the caruncle- cotyledon bond in retained fetal membrane. Injection of 1 L saline containing 200,000 IU of bacterial collagenase into the umbilical arteries of retained placentas caused earlier placental release than untreated contemporaries (Sheldon et al., 2006).

If applied within 24 to 72 h after calving, collagenase treatment can cause release of membranes in cows within 36 h. This treatment is targeted specifically at correcting the lack of cotyledon proteolysis and might be more effective than traditional therapies (Eiler and Hopkins, 1992). In general, manual removal and giving of intrauterine antimicrobial have been practiced to treat retained placenta (Sheldon et al., 2006).

Vitamin supplementation like antioxidants, vitamin E and selenium by single IM injection at week 3 prepartum is used as prophylactic dose to avoid placental retention in cows. The hormonal treatment of ROP is done by administration of prostaglandin, oxytocin and other steroidal hormones. PGF<sub>2</sub>α does not cause detachment of retained membranes, but it can improve reproductive performance in the early postpartum cow due to the uterokinetic effect (Gupta et al., 2005; Hauguel-de Mouzon and Guerre-Millo, 2006). Glucocorticoids (dexamethasone) could have a direct inhibition effect on collagenase activity which inhibits PGF<sub>2</sub>α synthesis within cotyledon cells (Gupta et al., 2005).

Oxytocin is the best hormonal choice for treatment of retained placenta in the early postpartum in animals. 20 IU three to four times daily have been used for retention of placenta, which acts as uterokinetic (Youngquist and Threlfall, 2006). Antimicrobial agent is also important in such treatment and it can treat secondary bacterial infection as well. The antibiotic required in the treatment of ROP are called intrauterine antimicrobial like tetracycline (powder 5 g) and procaine penicillin G intramuscular (IM) on day 1 to 3 which is used in endometritis (Youngquist and Threlfall, 2006). The treatment of retained placenta has been carried out by many clinicians and veterinarians; they used different methods such as manual removal, hormonal and non-hormonal like intrauterine antimicrobial and the like. This paper stands to illustrate the best treatment for ROP in remote areas; places where there are no clinics or drugs available, the drug for treatment of retained placenta is prepared from natural plant species.

#### **Plant source of medicine used for treatment of retained placenta in ruminants**

Plants are sources of various chemicals and secondary metabolites which have medical value in human and animal health. In the traditional way, the farmers treat their animals by administration of plant extract juice in patient animals. The herbal or plant prescriptions for treatment of retention of placenta are characterized as antibiosis, antiphlogosis and immune enhancement and fertility improvement without endometrial injuries. The survey data collections were conducted in Oromia region. The following plants have been used for treatment of ROP in Ethiopia: Flax seed (*Ija talbaa*), *Vernonia amygdalia* (Eebicha), *Dodonea angustifolia* (Itacha/Hitacha), *Ensete ventricosum*(Warqee), *Grewa ferrugina*, *Solanum acaule* (Iddii hoolaa), *Solanum acuminatum*(Iddii saree), *Dovyalis* spp. (Koshommii), *Galinsoga quadriradiata* (Waasee), *Plumbago zeylanica*, *Momordica* spp. (Qorii simbiraa), *Colocasia esculenta* (Goodarree), *Bryophyllum pinnatu* (Bosoqqee) and *Urera hypselodendron* (Laanqessaa) summarized in Table 1. These plants are sources of secondary chemical metabolites which act as antioxidant, antimicrobial and anti-inflammatory used to relief animal from pain and removal of placenta. In Ethiopia, especially in Oromia region, plants part like roots, leaves, stems, barks and seeds are collected and dried in order to prepare the extraction of chemical in form of powder and juice. The collected plants are used for treatment of ROP with a combination of other plants in order to increase the effectiveness of chemical. The combination of two different plants extract is better than extract chemical from a single plant. The following plants are common in the treatment of ROP. The preparation methods are like the root of *Momordica* spp. (*Qorii simbiraa*) which is

**Table 1.** The common plants used for treatment of retained placenta in Ethiopia, Oromia.

Local name (AfaanOromoo)	Scientific name	Family name	Part of plant	Use and route of administration
Leemmana	<i>Bambusa vulgaris</i>	Poaceae	Leaf	The feeding leaves of <i>Bambusa vulgaris</i> mixed with <i>Qunda barbarea</i> helps in expulsion of placenta. The leaves is dried and mixed with water, given to cattle orally ( <a href="http://www.hillagric.ac.in">http://www.hillagric.ac.in</a> )
Eebicha	<i>Vernonia amygdalia</i>	Asteraceae	leaf	Leaves of <i>Vernonia amygdalia</i> used treat ROP by mixing with table salt, and then administer to cow orally (Yigezu et al., 2014)
Talbaa	<i>Linum usitatissimum</i>	Linaceae	Seed	The powder of flax seed is mixed with water or salt, and then given to animals orally
Kosorruu adii	<i>Argemone mexicana</i>	Papaveraceae	Root	The powdered part is mixed with water, then administer to animals (Yadav et al., 2014)
Adamii (isabattee)	<i>Opuntieficus indica</i>	Cactaceae	leaf	Chopping the leaves and mixing with water and administering to animal orally with balanced dose (Bobaso, 2016).
Itacha/Hitacha	<i>Dodonea angustifolia</i>	Sapindaceae	leaf	The leaves is powdered and administer to animals orally, the chemical extraction increases the contraction of uterus which facilitates the expulsion of placenta (Tekle, 2014)
Warqee (Enset, kocho)	<i>Ensete ventricosum</i>	Musaceae	Leaves	Leaf of <i>Enset</i> is given for animals, and then the animal may relief from ROP (Mekonnen et al., 2016).
Qobboo	<i>Ricinus communis</i>	Euphorbiaceae	Leaves, seed	The dried part of the <i>Ricinus communis</i> is mixed with water and given for cow to repulsion of placenta (Birhanu et al., 2015).
Koshommii	<i>Dovyalis</i> spp.	Flacourtiaceae	Leaf	Leaf of <i>Dovyalis</i> is chopped and mixed with hot water and given for animal orally (Tolossa et al., 2013)
Qorii simbiraa	<i>Momordica</i> spp.	Cucurbitaceae		The root of <i>Momordica</i> spp is collected, dried ground and mixed with the powdered root of <i>Colocasia esculenta</i> , then this mixed powder is soaked in warm water and one cupful filtrate drenched into animals (Tolossa et al., 2013)
Bosoqqee	<i>Bryophyllumpinnatu</i>	Crassulaceae	Leaf	The latex part of leave or stem is mixed with <i>Urera hypselodendron</i> ( <i>Laanqessaa</i> ) collected and applied into intrauterine of cow (Tekle, 2014).

collected, dried, ground and mixed with the powdered root of *Colocasia esculenta*, and then this mixed powder is soaked in warm water and one cupful filtrate drenched into animals. After 24 to 48 h, the placenta would be ejected from the suffered animal. *Dovyalis* spp (*Koshommii*) is used to remove retained placenta, the leaf will be chopped and mixed with hot water and given to the animal orally (Tolossa et al., 2013).

In rural areas where modern medicine is inaccessible to farmers, Ethnoveterinary medicine (EVM) is often used to expel retained placenta in livestock. Ethnoveterinary medicine is a scientific term for traditional animal health care that encompasses the knowledge, skills, methods,

practices, and beliefs about animal health care found among community members (McCorke, 1986).

Ethnoveterinary medicine is the community based local knowledge and methods in caring, healing and management of livestock in remote area where modern medicine is limited, and it has been developed through some trial and errors, and experiments (Misra and Kumar, 2004). Ethnoveterinary medicine is sustainable and ecologically sound because plant products with recognized medicinal properties are far more accessible to the villagers than Western medicine. The EVM is a cheap and easy accessible alternative to expensive pharmaceuticals, this help the farmer make it available

at low cost or no cost at all (McGaw and Eloff, 2008).

The traditional remedies which have been used in treatment of retained placenta throughout the world are like salty water, soap detergent solution, *Terminalia serecea* roots, *Spirostachys africanum* bark and *Burkeaa fricanum* bark. Other herbal plants included *Ziziphus mucronata*, *Peltophorum africanum*, *Elephantorrhiza elephantina*, *Pouzolzia mixta*, *Dicerocaryu meriocarpum*, *Asparagus* spp., *Hermaniaguerkeana*, *Ozoroapaniculosa*, *Scacdoxus* spp. and *Boscia albitrunca* (Moreki et al., 2012). The remedies are administered either as a decoction or infusion. In the present study, the common plant parts used for treatment of retained placenta are barks, roots and bulbs and parts and products of animals such as skins and hides, bones, milk, butter and even urine and dung, are ingredients of Ethnoveterinary medicine (Balaji and Chakravarthi, 2010).

### Methods of preparation and administration of medical plant for animals

The preparations of these plants are, first collection of the leaves, roots and barks of the plant, then extraction of chemical in form of juice and powder, after which they are mixed with each other. After the plant extraction is prepared, the extraction is administered into the animals orally or through vaginal application of animals; this is done to detach the fetal membrane from its attachment. As the data were collected from community, they are informed that the methods of drug preparation from plants are concluded as follow; first the collection and identification of the plant, then drying the plant part like leaf, stem and root by putting it under shade and making it into powder. Afterward it is mixed with water and if available with salt water, then administered to animals through oral and intrauterine. The retained placenta would be removed from animal after 2 to 3 days. The most common plant which have been in use in Oromia region is flax seed (*Linum usitatissimum*). This flax seed is first grinded to form powder, then mixed with water and if available salt water (NaCl). After everything is prepared from powder of flax seed directly administering into animal orally. After one day the placenta will be removed from dairy cow and ewes. The common plant parts which are used in preparation of drug are leaves, stems, seeds, roots, barks and cell wall combs.

### The most common plant used for treatment of retained placenta in Ethiopia

#### *Dodonaea viscosa*

The origin of *Dodonaea viscosa* is believed to be Australia, but, currently it is distributed in the tropics and subtropics part of the world. The plant *Dodonaea viscosa*

(*D. viscosa*) is distributed to Africa (Ethiopia and others), Asia, Australasia, North America, and South America (Rani et al., 2009). *D. viscosa* is a shrub that grows to 3 meters tall and is indigenous in most Ethiopia regions like Oromia. Ethnopharmacology reporting in different parts of the world indicates a variety of therapeutic uses. The extracts leaves of this plant exhibited antibacterial and antioxidant properties (Riaz et al., 2012; Teffo et al., 2010).

*D. viscosa* acts as an antioxidant, antimicrobial, anti-inflammatory, induce wound healing and antispasmodic effect (Riaz et al., 2012; Teffo et al., 2010; Khan et al., 2012; Salinas-Sánchez et al., 2012). The chemical extract (alkaloids, tannins, phenols, gums and mucilage, fixed oils and fats, saponins, proteins, volatile oils, flavonoids and steroids) from *D. viscosa* by ethanolic extract, n-hexane, dichloromethane, ethylacetate, n-butanol, Chloroform, Ethanol and methanol crude extract from stem, bark and leaves act as antimicrobial. Gram positive bacteria were more sensitive to the extract of *D. viscosa* than Gram negative bacterium (Khurram et al., 2009; Ansar et al., 2013). Chopped leaves of *Dodonaea angustifolia* (*Hittacha in AfaanOromo*) mixed with water are filtered for treatment of retained fetal membrane (Tekle, 2014).

The plant contains many flavonoids: alizarin (5,7,4'-trihydroxy-3'-(3-hydroxymethylbutanol) 3,6-dimethoxy flavone), pinocembrin (5,7-dihydroxy flavanone), penduletin, (5,4'-Dihydroxy-3,6,7-trimethoxy flavone); isokaempferide (3,5,7,4'-tetrahydroxy-3'-methoxy flavone). 5,7-dihydroxy-3'-(4"-acetoxo-3"-methylbutyl)-3,6,4'-trimethoxy flavones; Kaempferol methyl ethers, 3, 5, 7-trihydroxy-4'-methoxyflavone; 5, 7, 4'-trihydroxy-3, 6-dimethoxyflavone; 5, 7-dihydroxy-3, 6, 4'-trimethoxyflavone (santin); 5-hydroxy -3, 7, 4'-trimethoxyflavone; 3,4',5,7-tetrahydroxy flavones (kaempferol). 5,7,4'-trihydroxy-3',5'-di(3-methylbut-2-enyl)-3,6-dimethoxyflavone and 5,7,4'-trihydroxy-3'-(4-hydroxy-3-methylbutyl)-5'-(3-methylbut-2-enyl)-3,6-Dimethoxyflavone; acacetin-7-Me ethers the flavonol-3-methyl ethers 4',5,7-trihydroxy-3,6-dimethoxyflavone, penduletin; 3, 6, 4'-trimethoxy-5,7-dioxyflavone; kaempferol 3,7-di-methyl ether and kaempferol-3,4',7-trimethyl ether were isolated from the aerial parts. Isorhamnetin and quercetin were isolated from the root bark of *D. viscosa*. Catechin or chromene groups, chalcones with trimethoxyphenyl group and tannin with 4-O-β-D-xylopyranoside were isolated from the leaves of *D. viscosa* (Abdel-Mogib et al., 2001; Muhammad et al., 2012; Niu et al., 2010; Teffo et al., 2010; Zhang et al., 2012). The chemical extraction mentioned above act as antioxidant and activation estrogen production. The chemical extraction also increases the contraction of uterus which facilitates the expulsion of placenta. The mode of action of *D. viscosa* extraction does not only act as antioxidant and muscle extraction, but also act as antimicrobial and anti-inflammatory which prevent shock and systemic disease from animals.

### ***Vernonia amygdalia***

*Vernonia amygdalia*, member of Asteraceae family, is a small shrub that grows in tropical Africa. *V. amygdalia* is a rapid regenerating soft wooded shrub of 2 to 10 m tall with petiolate leaves of around 6 mm in diameter. *V. amygdalia* is valuable medical plant that is distributed throughout Africa (Ijeh and Ejike, 2011; Yeap et al., 2010). It is used as active anti-cancer, antibacterial, antimalarial and antiparasitic agent (Clement et al., 2014; Yeap et al., 2010). As one study revealed, the leaf extracts of *V. amygdalia* have hypoglycaemic and hypolipidaemic properties in experimental animals and is used in managing diabetes mellitus (Iwara et al., 2015).

The common name of this plant varies in ethnic groups around world. In Ethiopia it is termed as 'Eebicha' in *Afaan Oromo*, in *Amharic* it is termed as 'Grawa'. The methods of chemical extraction from leaves, stem and bark of *V. amygdalia* has been reported in many researches. In the traditional way, the community prepares the extraction by grinding and filtering the leaves of the plant.

First step is collection of the plant parts, then keeping it dry and after that, they make powder and mix with water, filtering with some filter paper or piece of cloth and administering to the animals. In the modern ways, first collection of the plant is submitted to the laboratory and the chemical is extracted using different procedure of chemical extraction (Odey et al., 2012).

The leaves of *V. amygdalia* has been used to treat retained placenta by mixing with table salt, then administered to cow orally, and after some time the animal is relieved from retained placenta (Birhanu and Abera, 2015; Tekle, 2014). The ethanolic leaf extract of *V. amygdalia* have secondary metabolite chemical which acts as antimicrobial such as terpenes, oxalate, coumarins, lignins, sesquiterpenes, phytate, tannins, saponins, flavonoid, cyanogenic glycoside, alkaloids, anthraquinone, steroid and phenol (Owoeye et al., 2010; Udochukwu et al., 2015). The antioxidant, antimicrobial, anti-inflammatory, antiseptic and disinfectant, antifungal, anticancer and antifouling effect of the chemical extracted from *V. amygdalia* is used to relieve the animals from retained placenta and other complicated diseases (Igwe et al., 2015).

### **Phenol 3, 5-bis (1, 1 dimethylethyl), palmitic acid (Hexadecanoic acid), 1, 2-benzenedicarboxylic acid, Di-n-octyl phthalate, 9, 12-octadecadienoic acid (linoleic acid) and oleic acid**

Flavonoids present in *V. amygdalia* are like luteolin, luteolin 7-O-beta-glucuronoside and luteolin 7-O-beta-glucoside; these possess antioxidant activity (Audu et al., 2012; Yeap et al., 2010). Therefore this paper stand to illustrate the effect of *V. amygdalia* in treatment

of retained placenta after postpartum by acting on the prevention of microorganism growth and as antioxidant. The action of chemical extract from *V. amygdalia* act as increasing uterine contraction by decreasing progesterone concentration, this increment of uterine contraction may enhance removal of retained placenta (Yeap et al., 2010; Kamatenesi-Mugisha, 2004).

There are so many plants which are important in the treatment of retained placenta in domestic animals and even in human beings. The chemical which extract from those plants act as antioxidant, antimicrobial and initiates the contractility of the uterus, thus placenta are easily detached and removed out of the uterus. It is suggested that the tannin produced from the plant act as astringent to facilitate the contractility of uterus and removal of placenta.

When the wall of the uterus undergoes contraction, there may be shrinkage of small blood vessels and villi and the fetal caruncle easily detached from cotyledon of maternal, as a result, expulsion of placenta may occurred. Tannins containing plants are effective where protection of underlying tissues by skin or mucosa is compromised as seen in the case of wounds and retained placenta. Tannins proteins complexation limits fluid loss and forms a physical barriers to further tissue insult (Bruneton, 1995). Astringent property of tannins has a physiological effect such as contraction of the uterus which might facilitate the removal of retained placenta. By shrinking of the small blood vessels, the capillary pressure is lessened and separation of fetal membranes occur (Bruneton, 1995; Manspeaker, 2007). *Ricinus communis* has been used to treat retained fetal membrane (Birhanu and Abera, 2015). In treating retained fetal membrane, the leaf of *Ensete ventricosum* is also given for cow (Mesfin et al., 2016).

The plant extract of *Grewia ferruginea* were useful for easy expulsion of placental membranes. The feeding of bamboo leaves (*Bambusa vulgaris*) mixed with black pepper (*Qunda barbarea*) helps in expulsion of placenta. Raspberry leaves when fed to pregnant mares during the last 45 days of gestation reduce the incidence of periparturient diseases viz prolonged labor, retained placenta (www.hillagric.ac.in). *Argemone Mexicana* (Kossorruuadii) is used to remove retained placenta. 100 g whole *Argemone mexicana* plant with any available local grass for feeding once a day is used for removal of retained placenta in cows (Yadav et al., 2014).

*Opuntia indica* (Adami ishee battee) and *Urera hypselodendron* is used for expulsion of placenta retention by chopping the leaves and mixing with water and administering to the animal orally (Bobaso, 2016; Tekle, 2014). The chemical extraction is being performed in laboratory by different methods. However the modern extractions of chemical from plant parts are not known at the remote area where modern laboratory is scant. The farmers were used to administer the traditional medicine

without any extraction, this paper stand to report how to treat the retained placenta and identification of which species of plant are used for treatment of retained placenta in Ethiopia. Generally in Ethiopia, so many medical plant species exist. Out of these plants, the most common medical plants are rich in secondary chemical metabolites, which act as antimicrobial, antiparasitic, antispasmodic, analgesic, anti-cancer and anti-fungal and etc. The discussed plants are not published in any journal, this paper is pioneer to this study, and the most common way in treatment of ROP by chemical extraction is from the mixed chemical of different plant in order to increase the broad spectrum effect of drug.

The broad spectrum effect of this extracted drug acts as an anti-inflammatory, antispasmodic, antioxidant and antimicrobial. The extraction from the mentioned plants also used in enhancing the production of estrogen, and increasing contraction of uterus which facilitate removal of retained fetal membrane. Actually, the community used to treat their animals by separating plant extraction, and mixing the extracted chemical with other different chemicals.

All plants are effective, but drug is more effective when extractions of different plants are mixed rather than single plant. The orally drenched is more effective than others, because the toxicity of this extracted chemical is biotransformed and absorbs into the system, thus it easily reaches the uterus wall. The intrauterine application is also effective, however it is not practiced much in community. Moreover, the intrauterine infusion of this extracted drug from the mentioned plants may causes some disorder, and the toxicity of that chemical may have effect on the fertility of animals. Therefore, from this research it can be said that the orally drenched of extracted chemical from Ethnoveterinary medicinal plant is better than intrauterine infusion in the treatment of ROP.

## Conclusion

Retained placenta is common in domestic animals like ruminants and equines. The most common causes of ROP are dystocia, caesarian section, maternal hypoimmunity, mal and unbalanced nutrition, stress, hereditary predispositions or infections, failure of uterine contraction. Retention of placenta in ruminant animals have negative influence on the economic development of developing country by decreasing milk production, the inflammation of uterus, fever, weight loss, longer calving intervals and dying of animals. The treatments for retained placenta have been operated by different methods like hormonal and non-hormonal methods. The plant medicine which is used for treatment of retained placenta in ruminant animals are listed as follow: *Terminalia serecea* roots, *Spirostachys africanum* bark and *Burkeea fricanum* bark. *Ziziphus mucronata*,

*Peltophorum africanum*, *Elephantorrhiza elephantina*, *Pouzolzia mixta*, *Dicerocaryu meriocarpum*, *Asparagaus* spp., *Hermania guerkeana*, *Ozoroa paniculosa*, *Scadoxus* spp., *Boscia albitrunca*, *Vernonia amygdalia* (Eebicha), *Dodonea angustifolia* (Itacha/Hitacha), *Ensete ventricosumis*, *Grewa ferrugina*, *Solanum acaule* (Iddii hoolaa), *Solanum acuminatum* (Iddii saree), *Dovyalis* spp. (Koshommii), *Galinsoga quadriradiata*, *Plumbago zeylanica*, *Momordica* spp. (Qorii simbiraa), *Colocasia esculenta* (Goodarree), *Bryophyllum pinnatum* (Bosoqqee), *Linum usitatissimum* seed (IjaTalbaa) and *Urera hypselodendron* (Laanqessaa). The effects of retained placenta in domestic animals are critical in increasing mortality rate and decrease of economy in developing country. Therefore, this paper leads and gives the solution for the treatment of ROP in developing countries or remote areas where modern medicine is not available and veterinary clinic is scant. This paper also tends to apply some concept and information for production of drug from the mentioned plants in future industry.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

## ACKNOWLEDGEMENT

Above all, the author would like to give Praise to the Almighty GOD (ELOHIM) through his SON, YESHUA HAMASHIACH (JESUS CHRIST) for his love and mercy. Secondly, He would like to express his deep love and appreciation for his family. Lastly, His thanks go to Jimma University, ICT Center of JUCAVM for the support given him regarding computer and internet access.

## REFERENCES

- Abdel-Mogib M, Basaif SA, Asiri AM, Sobahi TR, Batterjee SM (2001). New clerodane diterpenoid and flavonol-3-methyl ethers from *Dodonaea viscosa*. *Die Pharmazie*, 56(10):830-831.
- Amin RU, Bhat GR, Ahmad A, Swain PS, Arunakumari G (2013). Understanding patho-physiology of retained placenta and its management in cattle-a review. *Veterinary Clinical Science*, 1(1):1-9.
- Ansar M, Ghulam M, Muhammad N (2013). Antibacterial and antifungal activity of *Dodonaea viscosa*(L.)Jacq.A wild plant of Azad Jammu and Kashmir. *International Journal of Biosciences (IJB)*, 3(9):1-7.
- Audu SA, Taiwo AE, Ojuolape AR, Sani AS, Bukola AR, Mohammed I (2012). A study review of documented phytochemistry of *Vernonia amygdalina* (Family Asteraceae) as the basis for pharmacologic activity of plant extract. *Journal of Natural Sciences Research*, 2(7):1-8.
- Azawi OI, Rahawy MA, Hadad JJ (2008). Bacterial isolates associated with dystocia and retained placenta in Iraqi buffaloes. *Reproduction in Domestic Animals*, 43(3): 286-292.
- Balaji N, Chakravarthi PV (2010). Ethnoveterinary practices in India- A review. *Veterinary World*, 3(12):549-551.
- Ball P, Peters A (2004). *Reproduction in Cattle*. Third Edition. Blackwell Publishing. P 430.



- Biner B, Bischoff M, Klarer F, Suhner F, Hüsler J, Hirsbrunner G. (2015). Treatment of Retained Fetal Membranes: Comparison of the Postpartum Period after Routine Treatment or Routine Treatment Including an Additional Phytotherapeutic Substance in Dairy Cattle in Switzerland. *Open Journal of Veterinary Medicine*, 5(04):93-99.
- Birhanu T, Abera D (2015). Survey of ethno-veterinary medicinal plants at selected HorroGudurru districts, Western Ethiopia. *African Journal of Plant Science*, 9:185-192.
- Bobaso H (2016). Survey of Veterinary Herbal Folk Medicine and Its Threats in West Arsi Zone, Oromia Regional State, Southern Ethiopia.
- Bruneton J (1995). Pharmacognosy, phytochemistry, medicinal plants. Lavoisier publishing.
- Clement E, Erharuyi O, Vincent I, Joy A, Christopher A, Anthony A, Onyekaba OJ, Iftikhar A, Abiodun F (2014). Significance of bitter leaf (*Vernonia amagdalina*) in tropical diseases and beyond: A Review. *Malar Chemother Cont*, 3(120):2.
- Davies CJ, Fisher PJ, Schlafer DH (2000). Temporal and regional regulation of major histocompatibility complex class I expression at the bovine uterine/placental interface. *Placenta*, 21(2-3):194-202.
- Davies CJ, Hill JR, Edwards JL, Schrick FN, Fisher PJ, Eldridge JA, Schlafer DH (2004). Major histocompatibility antigen expression on the bovine placenta: its relationship to abnormal pregnancies and retained placenta. *Animal reproduction science*, 82:267-280.
- Drillich M, Klever N, Heuwieser W (2007). Comparison of two management strategies for retained fetal membranes on small dairy farms in Germany. *Journal of dairy science*, 90(9):4275-4281.
- Drillich M, Mahlstedt M, Reichert U, Tenhagen BA (2006). Strategies to improve the therapy of retained fetal membranes in dairy cows. *Journal of dairy science*, 89(2): 627-635.
- Eiler H, FECTEAU KA (2007). Retained placenta. In *Current Therapy in Large Animal Theriogenology (Second Edition)* (pp. 345-354).
- Eiler H, Hopkins FM (1992). Bovine retained placenta: effects of collagenase and hyaluronidase on detachment of placenta. *Biology of reproduction*, 46(4):580-585.
- Eiler H, Hopkins FM (1993). Successful treatment of retained placenta with umbilical cord injections of collagenase in cows. *Journal of the American Veterinary Medical Association*, 203(3):436-443.
- Engler H, Bailey MT, Engler A, Sheridan JF (2004). Effects of repeated social stress on leukocyte distribution in bone marrow, peripheral blood and spleen. *Journal of Neuroimmunology*, 148:106-115.
- Entrican G, Wheelhouse N (2006). Immunity in the female sheep reproductive tract. *Veterinary Research*, 37:295-309.
- Fecteau KA, Eiler H (2001). Placenta detachment: Unexpected high concentrations of 5-hydroxytryptamine (serotonin) in fetal blood and its mitogenic effect on placental cells in bovine. *Placenta*, 22(1):103-110.
- Frazer GS (2005). A Rational Basis for Therapy in the Sick Postpartum Cow. *Veterinary Clinics of North America. Food Animal Practice*, 21:523-568.
- Fuchs AR, Ivell R, Ganz N, Fields MJ, Gimenez T (2001). Secretion of oxytocin in pregnant and parturient cows: corpus luteum may contribute to plasma oxytocin at term. *Biology of Reproduction*, 65(4):1135-1141.
- Fuchs AR, Rust W, Fields MJ (1999). Accumulation of cyclooxygenase-2 gene transcripts in uterine tissues of pregnant and parturient cows: stimulation by oxytocin. *Biology of reproduction*, 60(2):341-348.
- Gupta S, Gupta H, Soni J (2005). Effect of Vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. *Theriogenology*, 64:1273-1286.
- Hanafi EM, Ahmed WM, El Khadrury HH, Zabaal MM (2011). An overview on placental retention in farm animals. *Middle-East Journal of Scientific Research*, 7(5): 643-651.
- Hauguel-de Mouzon S, Guerre-Millo M. (2006). The placenta cytokine network and inflammatory signals. *Placenta*, 27(8):794-798.
- Hehenberger EM, Doherr MG, Bodmer M, Steiner A, Hirsbrunner G (2015). Diagnosis and therapy of retained fetal membranes, puerperal metritis and clinical endometritis in cattle: Results of the Online-survey among Swiss practitioners. II. Puerperal metritis and clinical endometritis. *Schweizer Archiv für Tierheilkunde*, 157(9):503-512.
- Igwe KK, Okafor II, Anika SM (2015). Phytochemical Characterization Of *Vernonia Amygdalina*. Del Ethanolic Extract Fraction And Contractile Response On Isolated Uterine Tissue In Female Albino Wistar Rats. *International Journal of Scientific Research and Management*, 3(11):3684-3693
- Ijeh II, Ejike CE. (2011). Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del. *Journal of Medicinal Plants Research*, 5(7):1051-1061.
- Iwara IA, Igile GO, Uboh FE, Eyong EU, Ebong PE (2015). Hypoglycemic and Hypolipidemic Potentials of Extract of *Vernonia calvoana* on Alloxan-Induced Diabetic Albino Wistar Rats. *European Journal of Medicinal Plants*, 8(2):78-86.
- Jemal JY (2016) A review on retention of placenta in dairy cattle. *International Journal of Veterinary Science*, 5(4):200-207.
- Joosten I, Hensen EJ (1992). Retained placenta: an immunological approach. *Animal Reproduction Science*, 28(1-4):451-461.
- Kaczmarowski MI, Malinowski ED, Markiewicz HA (2006). Some hormonal and biochemical blood indices in cows with retained placenta and puerperal metritis. *Bulletin-Veterinary Institute in Pulawy*, 50(1):89.
- Kaczmarowski MI, Malinowski ED, Markiewicz HA (2006). Some hormonal and biochemical blood indices in cows with retained placenta and puerperal metritis. *Bulletin of the Veterinary Institute in Pulawy*, 50:89-92.
- Kamatenesi-Mugisha, M (2004). Medicinal plants used in reproductive health care in Western Uganda: documentation, phytochemical and bioactivity evaluation (Doctoral dissertation, Ph. D. Thesis in Botany, Makerere University, Kampala, Uganda).
- Khan AZ, Mohammad A, Iqbal Z, Anis I, Shah MR, Nadeem S, Rabnawaz M, Shahidullah A, Khan H, Khan I (2012). Molecular docking of viscosine as a new lipoxygenase inhibitor isolated from *Dodonaea viscosa*. *Bangladesh Journal of Pharmacology*, 8(1):36-39.
- Khurram M, Khan MA, Hameed A, Abbas N, Qayum A and Inayat H (2009). Antibacterial activities of *Dodonaea viscosa* causing contact bioautography technique. *Molecules*, 14:1332-1341.
- Kimura K, Goff JP, Kehrl ME, Reinhardt TA. (2002). Decreased Neutrophil Function as a Cause of Retained Placenta in Dairy Cattle. *Journal of dairy science*, 85(3): 544-550.
- Laven RA, Peters AR (1996). Bovine retained placenta: aetiology, pathogenesis and economic loss. *The Veterinary Record*, 139(19):465-471.
- Maj JG, Kankofer M (1997). Activity of 72-kDa and 92-kDa matrix metalloproteinase in placental tissues of cows with and without retained fetal membranes. *Placenta*, 18(8):683-687.
- Manspecker JE (2007). Retained placentas. *Dairy Integrated Reproductive Management*, University of Maryland. <http://www.wvu.edu/~exten/infores/pubs/livepoul/dirm21.pdf>.
- McCorke CM (1986). An introduction to ethnoveterinary research and development. *Journal Ethnobiology*, 6:129-149.
- McGaw LJ, Eloff JN (2008). Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *Journal of Ethnopharmacology*, 119(3):559-574.
- Mesfin MM, Fitsum T, Melese Y, Tewodros G, Mebratu A (2016). Documentation of Ethno Veterinary Practices in Selected Sites of Wolaita and Dawuro Zones, Ethiopia. Available at: <https://globaljournals.org/item/6165-documentation-of-ethno-veterinary-practices-in-selected-sites-of-wolaita-and-dawuro-zones-ethiopia>
- Misra KK, Kumar KA (2004). Ethno-veterinary practices among the Konda Reddi of East Godavari district of Andhra Pradesh. *Studies of Tribes and Tribals*, 2(1):37-44.
- Moreki JC, Tshireletso K, Okoli IC (2012). Potential use of ethnoveterinary medicine for retained placenta in cattle in Mogono, Botswana. *Journal of Animal Production Advances*, 2(6):303-309.
- Muhammad A, Anis I, Khan A, Marasini BP, Choudhary MI, Shah MR (2012). Biologically active C-alkylated flavonoids from *Dodonaea viscosa*. *Archives of Pharmacol Research*, 35(3):431-436.
- Niu HM, Zeng DQ, Long CL, Peng YH, Wang YH, Luo JF, Wang HS, Shi YN, Tang GH, Zhao FW (2010). Clerodaneterpenoids and prenylated flavonoids from *Dodonaea viscosa*. *Journal of Asian Natural Products Research*, 12(1):7-14.
- Odey MO, Iwara IA, Udiba UU, Johnson JT, Inekwe UV, Asenye ME,

- Victor O (2012). Preparation of plant extracts from indigenous medicinal plants. *International Journal Science Technology*, 1:688-692.
- Owoeye O, Yousuf S, Akhtar MN, Qamar K, Dar A, Farombi EO, Onwuka SK, Choudhary MI (2010). Another anticancer elemanolide from *Vernoniaamygdalina* Del. *International Journal of Biological and Chemical Sciences*, 4(1).
- Rani MS, Pippalla RS, Mohan K (2009). An overview of *Dodonaea viscosa* Linn. *Asian Journal of Pharmaceutical Research and Health Care*, 1:97-112.
- Riaz T, Abbasi AM, Shahzadi T, Ajaib M, Khan MK (2012). Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscosa*. *Journal of the Serbian Chemical Society*, 77(4):423-435.
- Salinas-Sánchez DO, Herrera-Ruiz M, Pérez S, Jiménez-Ferrer E, Zamilpa A (2012). Anti-inflammatory activity of hauriwaic acid isolated from *Dodonaea viscosa* leaves. *Molecules*, 17(4):4292-4299.
- Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO (2006). Defining postpartum uterine disease in cattle. *Theriogenology*, 65(8):1516-1530.
- Sordillo LM, Aitken SL (2009). Impact of oxidative stress on the health and immune function of dairy cattle. *Veterinary immunology and immunopathology*, 128(1):104-109.
- Swiefy AS (2003). Effect of retained placenta on postpartum reproduction performance of Frisian cows. *Egyptian Journal of Animal Production*, 40:111-121.
- Tagesu A (2018). Review on the Reproductive Health Problem of Dairy Cattle. *Journal of Dairy and Veterinary Sciences*, 5(1):555655.
- Teffo LS, Aderogba MA, Eloff JN (2010). Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. Var. *angustifolia* leaf extracts. *South African Journal of Botany*, 76(1):25-29.
- Tekle Y (2014). An ethno-veterinary botanical survey of medicinal plants in Kochore district of Gedeo Zone, Southern Nations Nationalities and Peoples Regional State (SNNPRs). Ethiopia. *Journal of Scientific and Innovative Research*, 3:433-445.
- Tolossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijk JG (2013). Ethno-medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, 9(1):32.
- Tucho TT, Ahmed WM (2017). Economic and Reproductive Impacts of Retained Placenta in Dairy Cows. *Journal of Reproduction and Infertility*, 8(1):18-27.
- Udochukwu U, Omeje FI, Uloma IS, Oseiwe FD (2015). Phytochemical analysis of *Vernoniaamygdalina* and *Ocimumgratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *American Journal of Research Communication*, 3(5):225-235.
- Ward JW, Wooding FB, Fowden AL (2002). The effects of cortisol on the binucleate cell population in the ovine placenta during late gestation. *Placenta*, 23(6):451-458.
- Wischnal A, Nishiyama-Naruke A, Curi R, Barnabe RC (2001). Plasma concentrations of estradiol 17 $\beta$  and PGF2 $\alpha$  metabolite and placental fatty acid composition and antioxidant enzyme activity in cows with and without retained fetal membranes. *Prostaglandins and other Lipid Mediators*, 65(2):117-124.
- Yadav SS, Bhukal RK, Bhandoria MS, Ganie SA, Gulia SK, Raghav TB (2014). Ethnoveterinary Medicinal plants of Tosham block of district Bhiwani (Haryana) India.
- Yeap SK, Ho WY, Beh BK, San Liang W, Ky H, Yousef AH, Alitheen NB (2010). *Vernoniaamygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bio-activities. *Journal of Medicinal Plants Research*, 4(25):2787-2812.
- Yigezu Y, Haile DB, Ayen WY (2014). Ethnoveterinary medicines in four districts of Jimma zone, Ethiopia: cross sectional survey for plant species and mode of use. *BMC Veterinary Research*, 10(1):76
- Youngquist RS, Threlfall WR (2006). *Current Therapy in Large Animal Theriogenology-E-Book*. Elsevier Health Sciences.
- Zhang LB, Ji J, Lei C, Wang HY, Zhao QS, Hou AJ (2012). Isoprenylated flavonoid and adipogenesis-promoting constituents of *Dodonaea viscosa*. *Journal of natural products*, 75(4):699-706.

*Full Length Research Paper*

# A study on gross and histopathological pulmonary lesions of cattle slaughtered at Abergelle Abattoir, Mekelle, Tigray, Ethiopia

Kidane Workelul Yalew<sup>1\*</sup>, Nesibu Awol<sup>2</sup>, Yishak Tsegay<sup>1</sup>, Haftay Abraha<sup>1</sup> and Hailesilassie W/mariam<sup>1</sup>

<sup>1</sup>Department of Veterinary Medicine, College of Veterinary, Mekelle University, Kelamino, Mekelle, Ethiopia.

<sup>2</sup>Department of Veterinary Medicine, School of Veterinary, Wollo University, Dessie, Ethiopia.

Received 12 February, 2018; Accepted 18 April, 2018

Animals in sub-Saharan African countries are used as source of milk, meat, hides and skin where animal diseases are one of the primary constraints in increasing the productivity of food animals. Studies were conducted to examine various respiratory problems of cattle with purpose of identifying the gross and histopathological lesions in lungs of slaughtered cattle at Abergelle Export Abattoir from April 2015 to June 2015 Mekelle, Tigray, Ethiopia. A total of 240 lungs of cattle originating from different parts of Tigray regions were examined and as many as 208 lung samples with gross pulmonary lesions were collected for further confirmatory histopathological study. The prevalence of pulmonary lesions of cattle in this study was 86.6% (n=208). The case of pneumonia was found to be highest followed by lung emphysema and congestion besides cases of oedema, hydatidosis, abscess and atelectasis. These results indicated that respiratory problem is still a major health problem in cattle production.

**Key words:** Lung, pneumonia, pulmonary lesion, Ethiopia.

## INTRODUCTION

Domestic animals in Ethiopia are mainly used as drought animals, source of milk, meat, hide and skin and as pack animals. Animal diseases are one of the primary constraints in increasing the productivity of food animals in sub-Saharan Africa (Lemma et al., 2001).

Lopez (2012) stated diseases affecting the respiratory system are generally the leading causes of morbidity and mortality in large domestic animals. The management and environmental stress are often the decisive factors for the development of clinical diseases (Howard, 1993).

Environmental and management stresses like over work, undue exposure to cold winds and rain, sudden change of climate, insufficient food and chronic diseases particularly trypanosomosis are responsible for respiratory diseases (Andrews and Kennedy, 1997; Dungworth, 1993). Pulmonary tissues are damaged by potential bacterial pathogens especially *Pastuerella* infection that normally reside in the upper respiratory tract (Carter, 1984). Even the lung tissues can be found infected with food borne parasitic zoonotic infections

\*Corresponding author. E-mail: kiduney06@gmail.com.

(Sandhu et al., 1995; Chhabra and Singla 2009).

The reaction of the lung tissue may be in the form of an acute fibrinous process as in pasteurellosis, a necrotizing lesion as infection with *Fusobacterium necrophorum* or as a more chronic caseous or granulomatous lesion in *Mycobacterial* or *Mycotic* infections (Radostits et al., 2007).

The gross and histopathological characterization of the pulmonary disease has not yet been studied in Mekelle and around slaughter at Abergelle Export Abattoir; for this reason this study is designed with the objective to assess the major pulmonary lesion of the slaughter cattle based on lobular distribution of the infected lung and to identify as well as characterize the types of gross and histopathological lesions in lungs of cattle slaughtered at Abergelle Export Abattoir.

## MATERIALS AND METHODS

### Study area

The study was conducted in Abergelle Export Abattoir, Mekelle. Mekelle is the capital city of Tigray Region. It is located around 780 km north of the Ethiopian capital City Addis Ababa, at a latitude and longitude of 13°29'N 39°28'E, with an elevation of 2084 m above sea level. The mean annual rainfall of the area is 628.8 mm. The annual minimum and maximum temperature is 11.8 and 29.94°C, respectively (RSTBARD, 2009).

### Study type and study animals

A cross-sectional study was undertaken to establish the prevalence of gross and histopathological pulmonary lesions of cattle slaughtered at Abergelle Export Abattoir, Mekelle. The study was conducted purposively on 240 cattle subjected to slaughter from different parts of Tigray region. All cattle examined in this study were male greater than six years of age and have good body condition were apparently healthy during ante-mortem inspection.

### Sample collection and processing

Immediately after slaughter, post-mortem examination was made by visual examination, palpation and incision for the presence of any lung lesion. The gross appearance, location as well as size of the lesions and observations made on incision were also recorded. Out of 240 cattle lungs examined, a total of 208 lung tissue samples with gross pulmonary lesions were collected for histopathological study. Tissue samples having a thickness of up to 2 to 3 cm were collected from every pulmonary lesion. They were taken from the margin of healthy and affected tissue parts and fixed in 10% buffered neutral formalin (BNF) immediately. Each sample was fixed separately and transported to pathology laboratory for processing. After fixing tissue samples for 24 h, all samples collected for histopathological study were dehydrated in alcohol, embedded in paraffin, sectioned at 4 to 6 µm thickness and stained with Haematoxyline - Eosin stain. The stained samples were examined using light microscope. Sample collection and processing was done as per the method described by Assegedech (2005) and Bancroft et al. (1996).

### Data analysis

The data was entered into Microsoft excel spread sheet and coded

appropriately. For data analysis, SPSS version 15 was used. In this data analysis, descriptive statistic was used to determine the proportion of the different respiratory lesions and their lobular distributions. Chi-square was used to test the lobular distributions of pulmonary lesions.

## RESULTS

### Pulmonary lesions

Out of the total 240 slaughtered cattle examined during the study, 208 cattle (86.6%) were found to have pulmonary lesions. On the basis of gross and microscopic examination pneumonia, pulmonary emphysema, pulmonary congestion and oedema, hydatidosis, pulmonary abscesses and atelectasis were identified with prevalence of 33.33% (n=80), 31.25% (n=75), 12.08% (n=29), 6.67% (n=16), 2.08% (n=5) and 1.25% (n=3), respectively. The frequency and prevalence of pulmonary lesions identified in this study was summarized in Table 1. The lobular distribution of pneumonia, pulmonary emphysema, and pulmonary congestion and edema were significantly ( $p$ -value = 0.000) higher in the right cranial lobe than the other lobes of lungs. However, pulmonary abscess was significantly ( $p$ -value = 0.000) higher in the right middle and caudal lobes of the lungs compared to the other lung lobes. Hydatidosis, was also most frequently encountered in the right caudal lobe ( $p$ -value = 0.000). In addition, atelectasis was most commonly encountered in the caudal lobes of both right and left lungs with  $p$ -value < 0.321. The lobular distributions of pulmonary lesions were summarized in Table 2. Representative images of some pulmonary lesions are indicated in Figure 1.

## DISCUSSION

Out of 240 cattle lungs examined, 86.6 % had pulmonary lesions. This result was in agreement with the report of Gebrehiwot et al. (2015) and Abayneh (1999) who reported pulmonary lesions of cattle with prevalence of 86.2 and 83.87%, respectively. However, the result of this study was higher than the findings of Amene et al. (2012) and Ahmed et al. (2013) who reported pulmonary lesions with prevalence of 46.22 and 44.6%, respectively. These all results showed that respiratory diseases are highly prevalent in cattle production area. This high prevalence of pulmonary lesions could be due to the older age of the animals at slaughter with possibility of exposure to one of the agents which causes respiratory disease through time at least once.

The overall prevalence of pneumonia in this study was 33.33%. This agree with the report of Ahmed et al. (2013) who reported pneumonia in cattle at a prevalence of 28.7% but higher than the results of Yifat (2011) and Amene et al. (2012) who reported 1.8 and 1.11%

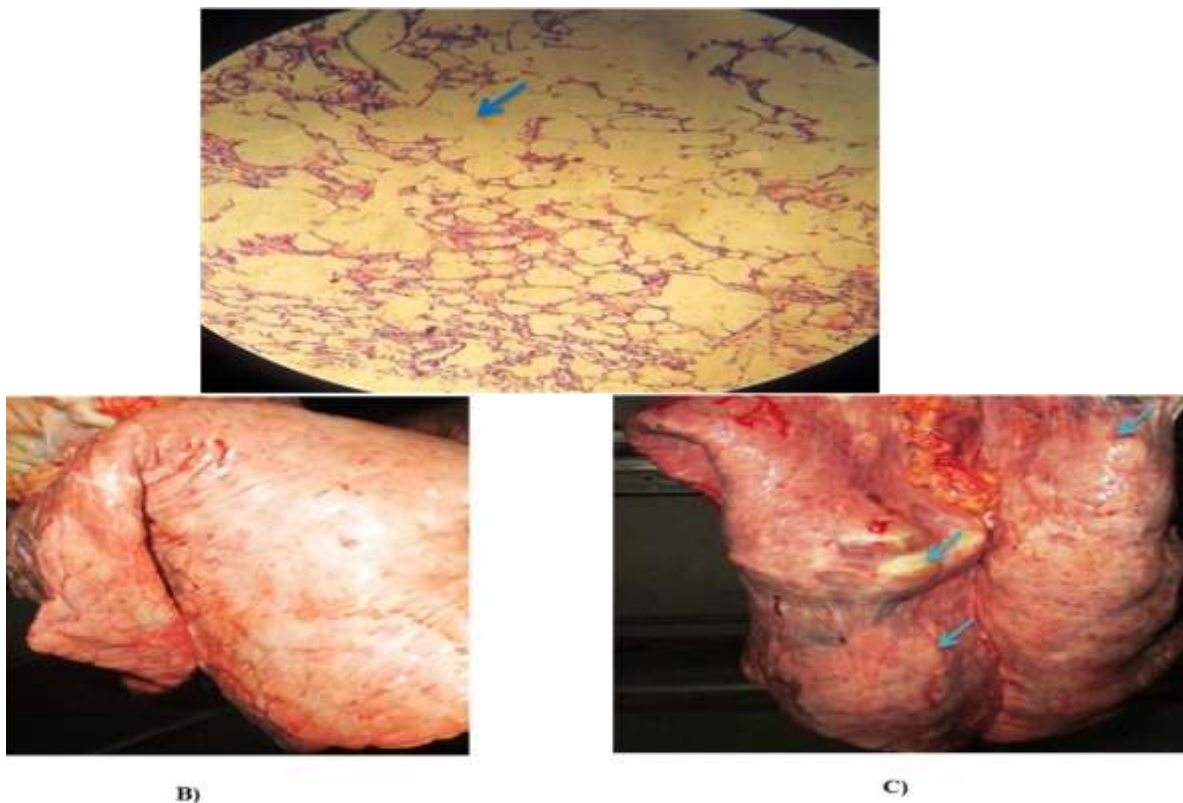
**Table 1.** Prevalence of pulmonary lesions in lungs of cattle slaughtered at Abergelle Export Abattoir, Mekelle.

Pulmonary lesion	Frequency	Prevalence (%)
Pneumonia	80	33.33
Emphysema	75	31.25
Pulmonary congestion and edema	29	12.08
Hydatidosis	16	6.67
Pulmonary abscesses	5	2.08
Atlectiasis	3	1.25

**Table 2.** Lobular distributions of pulmonary lesions.

Lesion	Percentage of distribution of pulmonary lesions (%) in different lobes of the lungs							Chi-square	
	RCC	RCD	RM	RD	LCC	LCD	LD	x <sup>2</sup>	P-value
Pneumonia	86.25	32.5	36.25	18.75	20	15	2.5	168	0.000
Emphysema	62.67	18.67	25.33	12	13.33	6.67	2.67	299	0.000
Pulmonary congestion and Oedema	72.41	17.24	27.59	31.03	20.69	17.24	3.45	104	0.000
Hydatidosis	6.25	18.75	31.25	62.5	0	6.25	25	61.1	0.000
Pulmonary abscess	0	20	40	40	0	20	20	17.4	0.008
Atelectasis	33.33	33.33	0	66.67	33.33	33.33	66.67	7	0.321

RCC, Right Cranial Cranial; RCD, Right Cranial Caudal; RM, Right Middle; RD, Right Caudal; LCC, Left Cranial Cranial; LCD, Left Cranial Caudal; LD, Left Caudal.



**Figure 1.** Microscopic and gross representative images of typical pulmonary lesions. (A) Emphysema with ruptured alveolar wall and coalescence of alveolar space (arrow); (B) Gross lesion of emphysematous lungs; (C) Multiple hydatid cysts in lungs (arrows).

prevalence of pneumonia in cattle, respectively. The factors affecting are different in management systems, breed of animals, nutrition, climatic factors, and animal health extension services etc.

Emphysema was found to be the second most prevalent lesion with a prevalence of 31.25%. This result coincides with the report of Gebrehiwot et al. (2015) where emphysema was observed on 36.3% of cattle and higher than the studies of Abayneh (1999) and Amene et al. (2012) who reported emphysema at prevalence of 16.53 and 6.77%, respectively. The emphysema could be due to excessive destruction of alveolar walls as a result of an imbalance between proteases produced by phagocytes and antiproteases produced in the lungs as a defense mechanism, secondary to obstruction of outflow of air or agonal at slaughter as indicated by Lopez (2012) and Dungworth (1993). Emphysema was encountered more frequently on the right cranial lobes of the lung.

Pulmonary oedema and congestion occurred at a prevalence of 12.08% and this was higher than the results of Amene et al. (2012) and Ahmed et al. (2013) who reported a prevalence of 2.33 and 6.5%, respectively. However, the result of this study was lower than the results of Rahman et al. (2003) and Gebrehiwot et al. (2015), who reported pulmonary oedema and congestion in cattle at a prevalence of 61.53 and 38.5%, respectively. Pulmonary oedema and congestion provides an ideal environment for the growth of pathogens of relatively low virulence.

Hydatidosis was encountered in 6.67% of the examined cattle lungs and lower than the findings of Rahman et al. (2003), Gebrehiwot et al. (2015) and Amene et al. (2012) who reported prevalence of 25, 18.3 and 35.88%, respectively. This difference in the prevalence of hydatidosis may be due to variation of dogs and wild carnivore population. Hydatid cysts were distributed most frequently on the right caudal lobes of the lungs. This could be due to the larger size of the caudal lobes, which in effect get a greater volume of blood supply (Lopez, 2012).

Pulmonary abscesses were encountered in 2.08% of the cases. This result was higher than the result of Amene et al. (2012) (0.11%) and lower than that of Gebrehiwot et al. (2015) (7.1%) and Ahmed et al. (2013) (9.4%). Pulmonary abscess arise from either focal residues of severe, supportive lobar or bronchopneumonia or from septic emboli lodging in the pulmonary vascular bed. The most common sources of septic emboli include ruptured hepatic abscess in cattle, suppurative metritis, mastitis, septic arthritis, omphalophebitis in farm animal and bacterial endocarditis (right side) in all species (Lopez, 2012; Dungworth, 1993). Additionally the two less common causes of pulmonary abscess are aspiration of foreign bodies and direct traumatic penetration of the lungs (Lopez, 2012; Dungworth, 1993).

The prevalence of atelectasis in this study was 1.25%.

Atelectasis is common when collateral ventilation is less (Lopez, 2012). The cause of usual atelectasis is occlusion of the bronchus or bronchiole, which supplies it. This results most often from a plug of mucus or purulent exudates. The air contained at the time the bronchus is closed is absorbed in a short time as is regularly the case with entrapped gases. The airless alveoli then collapse under surrounding pressures (Lopez, 2012; Jone and Hunt, 1983). It also accompanies space occupying lesions in the thoracic cavity or on the lung parenchyma like neoplasia, granuloma, hydatid cyst etc and the accumulation of transudate and exudates when their volume is large (Lopez, 2012; Jone and Hunt, 1983; Dungworth, 1993). In all types of atelectasis the collapsed lung is prone to secondary infections (Lopez, 2012).

## CONCLUSION AND RECOMMENDATIONS

Out of the total 240 lungs of cattle examined, 86.6% were found with one or more pulmonary lesions, indicating pulmonary diseases as an important constraint of cattle production. The lesions encountered in this study will also play a role as a predisposing factors for respiratory disease outbreak under the influence of stress factors such as environmental change, extremes of climatic conditions, transportation and shortage of feeds and water or alone. Therefore, the prevailing environmental condition undue exposure to cold winds and rain, sudden change of climate coupled with the management stresses like over work might reverse these hidden inactive lesions and thereby contribute for the higher occurrence of respiratory diseases in cattle's.

Taking these facts into consideration the following recommendations are forwarded:

1. Further and subsequent study is recommended on a larger scale with specific data about the origin of animals slaughtered.
2. Awareness of the public about hydatidosis should be increased through public education and infected organs should be properly disposed.
3. Strong collaboration among governmental organization, nongovernmental organization, veterinarian (researchers) and livestock owners should be made in order to determine the impact of respiratory disease on cattle production and to design control and prevention strategies.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENT

The author sincerely would like to appreciate Mekelle

University College of Veterinary Medicine for provision of the laboratory and other facilities. They also extend their thanks to the Abergelle Export Abattoir for providing their facility to conduct the study.

## REFERENCES

- Abayneh L (1999). Pulmonary lesions of cattle slaughtered at Assela abattoir, Ethiopia. DVM Thesis, AAU, FVM, Debre Zeit, Ethiopia, pp. 1-21.
- Ahmed AM, Ismail SAS, Dessouki AA (2013). Pathological lesions survey and economic loss for male cattle slaughtered at Ismailia abattoir. *Inter. Food Res. J.* 20(2):857-863.
- Amene F, Eskindir L, Dawit T (2012). The cause, rate and economic implication of organ condemnation of cattle slaughtered at Jimma municipal abattoir, Southwestern Ethiopia. *Glo. Vet.* 9(4):396-400.
- Andrews GA, Kennedy GA (1997). Respiratory diseases in diagnostic pathology Veterinary Clinical. North America: Food Animal. *Practies.* 13(3):515-547.
- Assegedech S (2005). Histopathology. In: Standard Veterinary Laboratory Diagnostic Manual, (Vol. V). Ministry of Agriculture and Rural Development, Animal Health Department. Addis Ababa, Ethiopia. pp. 1-60.
- Bancroft JD, Stevens A, Turner DR (1996). Theory and practice of histological techniques. 4th Ed. Churchill Livingstone, Edinburgh, London, Melbourne, New York.
- Carter GR (1984). Diagnostic Procedures in Veterinary Bacteriology and Mycology, 4th ed. Charles C. Thomas, Springfield, Illinois, U.S.A. pp. 3-166.
- Chhabra MB, Singla LD (2009). Food-borne parasitic zoonoses in India: Review of recent reports of human infections. *J. Vet. Parasitol.* 23(2):103-110.
- Dungworth DL (1993). The respiratory system. In: K.V.F. Jubb, P.C. Kennedy and Palmer (eds), *Pathology of Domestic Animals 2*. Academic Press, INC. Harcourt Brace Jovanovich, Publishers, San Diego, U.S.A. pp. 539-699.
- Gebrehiwot T, Verma PC, Berhanu H (2015). Study on gross pulmonary lesions in lungs of slaughtered animals and their economic importance in Tigray, Ethiopia. *Momona Ethiop. J. Sci.* 7(1):46-54.
- Howard JL (1993). *Current Veterinary therapy 3rd ed: Food Animal practice*. Saunders company, Philadelphia, U.S.A. pp. 633-642.
- Jone TC, Hunt RD (1983). The respiratory system. In : *Veterinary pathology, 5<sup>th</sup> (ed)*. Lea and Febiger, Philadelphia, U.S.A. pp. 1208-1249.
- Lemma M, Kassa T, Tegen A (2001). Clinically manifested major health problems of cross breed dairy herds on urban and peri-urban production system on the central high lands of Ethiopia. *Trop. Anim. Health and Prod.* 6:34-43.
- Lopez A (2012). Respiratory system. In Cariton, W.W., Mc Gavin, M.D. (eds), *Thomson's Special Veterinary pathology*. 5th ed, Chicago pp. 116-174.
- Radostiis OM, Gay CC, Blood DC, Kenneth H (2007). *Veterinary Medicine- Text Book of the Diseases of cattle, sheep, pig, goats and horses*. 9th edition, W.B. Saunders, Tottenham Court Road, London, pp. 431-441.
- Rahman A, Nooruddin M, Begum N, Lee J (2003). Epidemiological study of pulmonary lesions and diseases in slaughtered cattle. *Korean J. Vet.* 26(1):81-88.
- Regional State of Tigray Bureau of Agriculture and Rural Development (RSTBARD) (2009). Estimated animal population of Tigray region.
- Sandhu BS, Brar RS, Singla LD, Singh H, Brar APS (1995). Sarcocystis in alveolar tissue of buffalo lungs. *Ind. J. Vet. Pathol.* 19(1):53.
- Yifat D (2011). Major causes of organ condemnation and financial significance of cattle slaughtered at Gondar Elfora Abattoir, Northern Ethiop. *Glob. Vet.* 7(5):487-490.



Full Length Research Paper

# Study on calf coccidiosis in dairy farms in and around Holeta Town, Finfine Zuria Liyu Zone, Oromia, Ethiopia

Zerihun Adugna Regasa<sup>1</sup>, Belay Mulatea<sup>1</sup> and Temesgen Kassa Getahun<sup>2\*</sup>

<sup>1</sup>Faculty of Veterinary Medicine and Animal Health, Wollo University, Ethiopia.

<sup>2</sup>Animal Health Research, Holeta Agricultural Research Center, Ethiopian Institute of Agricultural Research, P. O. Box 31, Holeta, Ethiopia.

Received 27 July, 2017; Accepted 19 October, 2017

Bovine coccidiosis is a protozoan infection caused by different species of *Eimeria* which has a worldwide distribution. The disease which mainly affects calves belongs to large herd size where hygiene is not well managed and is associated with poor body condition. A cross-sectional study was conducted from November 2016 to April 2017 to determine the prevalence of bovine coccidiosis and identify the associated risk factors in semi intensive and extensive dairy farms in and around Holeta town, Finfine Zuria Liyu Zone, Oromia Regional State, Ethiopia. Fecal samples were randomly collected from three hundred and eighty four calves belonging to dairy farms and examined for the presence of the oocysts of *Eimeria* by floatation technique using saline solution. The study revealed that the overall prevalence of coccidiosis was 26.04%. The risk factors considered were age, sex, breed, production system, herd size, fecal consistency, body condition and hygienic status of the house. The prevalence of coccidiosis was higher within calves in poor hygienic (58.6%) dairy farms than calves from better hygienic farms (14.6%). There was also significant difference ( $P<0.05$ ) in the prevalence of coccidiosis between different herd sizes with higher prevalence in herd size  $>10$  animals (39.3%). The highest prevalence of coccidiosis was recorded in calves with diarrheic faeces (91.7%) than calves with soft, constipated and normal fecal consistency ( $P<0.05$ ). Appropriate monitoring and control of the disease is advisable in the study farms.

**Key words:** Calf, coccidiosis, dairy farms, *Eimeria*, Holeta, prevalence.

## INTRODUCTION

Ethiopia is endowed with abundant livestock resources of varied and diversified genetic roles with specific adaption to its wide range of agro ecologies (CSA, 2015). This great livestock potential is not properly exploited due to many prevailing socio economic values and attitudes,

traditional management methods, limited genetic potential and rampant disease.

Gastrointestinal parasite infections are a problem for both small and large scale farms; however, their impact is greater in sub-Saharan Africa. The prevalence of

\*Corresponding author. E-mail: [Temesgen.kassa@yahoo.com](mailto:Temesgen.kassa@yahoo.com). Tel: +251933864380. Fax: 0112370377.



gastrointestinal parasites and the severity of infection vary considerably depending on the genera of helminth and protozoan parasites involved, animal species, local environmental conditions such as humidity, temperature, rainfall, vegetation and management practices (Debela, 2002; Sandhu and Singla, 2005; Wadhawa et al., 2011).

The most important disease problems in the young calf are pneumonia and diarrhea. The pathogens associated with calf diarrhea are rotavirus, corona virus, *Salmonella* species, protozoan parasites, *Eimeria* and *Cryptosporidium* species (Bhat et al., 2012; Brar et al., 2017). Bovine coccidiosis is a protozoan disease of the intestinal tract caused by microscopic organisms called coccidia; and is one of the most common and important disease of cattle worldwide (The Merck Veterinary Manual, 2005). This disease is usually the most known and devastating protozoan disease in calves under age of one year (Ernst et al., 1987).

Previous works which were conducted in different parts of Ethiopia showed that coccidiosis is a paramount important protozoan disease in younger calves (<12 months) which were kept in poor hygienic status as well as improperly nourished with colostrum (Abebe et al., 2008; Mehreteab et al., 2012; Alemayehu et al., 2013; Temesgen, 2016). Although, coccidiosis is an important cause of calf morbidity and mortality in Ethiopia in general, and in the study area in particular, there is no previous detail information on its prevalence coccidiosis in the study area.

Therefore, this study was conducted to determine the prevalence and associated risk factors of calf coccidiosis in farms in and around Holeta, Finfine Zuria Liyu zone, Ethiopia.

## MATERIALS AND METHODS

### Study area

The present study was conducted in extensive and semi intensive farms found in and around Holeta town located in Finfine Zuria Liyu Zone, Oromia Regional State, Ethiopia during the period between November 2016 and April 2017 to determine calf coccidiosis and its putative risk factors. Holeta is located 45 km west of Addis Ababa at altitude 2400 m above sea level. It is geographically located between 9° 3'N latitude and 38° 30' E longitudes. The area experienced bimodal rainfall pattern with a short rainy season from February to April and the long rainy season from the middle of June to end of September. The remaining months are dry periods. The area gets an annual rain fall of 1000 to 1100 mm and the annual temperature ranges between 18 and 24°C. The total cattle population of the study area is estimated to be 175,741, out of which 172,769 (98.3%) heads of cattle are local breeds and 2972 (1.7%) are crosses kept under extensive and semi intensive management systems (WoWAHA, 2015).

### Study animals

The study was carried out on 384 calves within the age of 1 month to 1 year old. The samples were randomly collected from calves

reared under semi intensive and extensive management systems. Examined calves were categorized based on their age and grouped into three as: group I (1 to 4 months age), group II (5 to 8 months) and group III (9 to 12 months of age), based on house hygiene grouped into three (good, moderate and poor) and also based on size of herd grouped into three (<10, 11-20 and >20 head of calves).

### Study design and sample size determination

The type of study was cross sectional with simple random sampling technique conducted between November 2016 and April 2017 to determine the prevalence and associated risk factors of calf coccidiosis in and around Holeta town. The desired sample size for this study was determined by using the single population proportion formula according to Thrusfield (2005). Since there is no previous report on calf coccidiosis in the study area, the sample size was established based on the 50% expected prevalence, 5% desired absolute precision and 95% confidence level (CI).

$$n = \frac{z * P_{ex}(1 - P_{ex})}{d^2}$$

Where, n = required sample size; z=1.96<sup>2</sup>; p<sub>ex</sub> = expected prevalence; d = desired absolute precision.

Thus, the desired sample size for P<sub>ex</sub> = 0.5 is n= 384 calves included in this study. While collecting faecal samples, data related to age, sex, breed, herd size management system, body condition, fecal consistency and hygienic status of barn were properly recorded.

### Faecal sample collection and examination

A total of 384 faecal samples were collected directly from the rectum of each sampled animal with strict sanitation, and placed in air and water tight sample vials. After collection, the samples were transported in ice box to Holeta Agricultural Research Center (HARC) Parasitological Laboratory for fecal examination using simple floatation technique with saline solution (Yu et al., 2011; Gupta and Singla 2012).

### Data management and analysis

All data collected were entered and managed in MS-Excel software program and analyzed using SPSS 20.0 statistical software version. Descriptive statistics such as percentage was used to approximate the prevalence of calf coccidiosis in the study area. Prevalence was calculated as number of positive calves harboring *Eimeria* oocysts divided by the total calves examined. Chi-square (χ<sup>2</sup>) statistics were used to test the association between variables. At p <0.05 was taken as statistically significant.

## RESULTS

### Overall prevalence

In the present study, out of total 384 samples tested, 100 (26.04%) were positive for the presence of *Eimeria* oocysts.

The prevalence was higher in Barfata (37.5%) as compared to other study localities (p <0.05) as shown in Table 1.

**Table 1.** Prevalence of calves Coccidiosis in the study localities.

Study locality	No. of animals examined	No. of positive	Prevalence (%)	$\chi^2$	p-Value
Holeta	206	37	18	15.698	0.000
Barfata	98	37	37.5		
WajituHarbu	80	26	32.5		
Total	384	100	26.04		

### Potential risk factors

The analysis of putative risk factors was made by Chi-square analysis. The result showed strong significant associations between coccidian infection and herd size ( $\chi^2 = 16.387$ ,  $P = 0.000$ ), different body condition scores ( $\chi^2 = 51.447$ ,  $P = 0.000$ ), faecal consistency ( $\chi^2 = 44.668$ ,  $P = 0.000$ ) and hygienic status of the house ( $\chi^2 = 29.393$ ,  $P = 0.000$ ). However, no significant difference was observed between the prevalence of coccidiosis and different age groups ( $\chi^2 = 3.363$ ,  $P = 0.186$ ), sexes ( $\chi^2 = 0.437$ ,  $p = 0.508$ ), breeds (0.103,  $P = 0.748$ ) and different management systems ( $\chi^2 = 0.281$ ,  $P = 0.596$ ) as shown in Table 2.

### DISCUSSION

Calf coccidiosis causes a significant economic loss through morbidity and mortality worldwide. The result of the present study conducted in three localities of Holota town and its environs showed an overall prevalence of 26.04% coccidian infection in calves. The 26.04% prevalence of coccidiosis in this study is in line with previous studies of Bekele et al. (2012) [22.7%] in Dire Dawa, Temesgen (2016) [24.3%] in South Wollo Zone Amhara region, Ethiopia, Toaleb et al. (2011) [24.2%] in Egypt. However, the prevalence result of the present study is much lower than many of the previous reports in Ethiopia, namely, 68.1% in Addis Ababa and Debre Zeit Dairy Farms (Abebe et al., 2008), 31.9% in Kombolcha, south Wollo (Alemayehu et al., 2013), 62.5% in Asella town (Ibrahim, 2016) and 38.9% in and around Asella town dairy farms (Tsegaye, 2016). The findings of this study are also much lower than that of other countries of the world. For instance, a prevalence of 47.1% was reported in China (Dong et al., 2012). The results of the present study, however, is higher than previous reports by Gillhuber et al. (2014) (13.3%) in Southern Germany, Hussin (2016) (9.5%) in Iraq and Das et al. (2015) (11.9%) in India. Such inconsistency in the prevalence rate of coccidiosis may be due to the variation in diagnostic tests, age of the animals, susceptibility of different breeds to the disease, stress level, handling, climatic and other factors of agro-ecology, variation in the study season, number and target group of the study animals and husbandry practices (Radostitis et al., 2007a; Abebe et al., 2008; Heidari et al., 2014).

This study showed variation in the prevalence of calf coccidiosis between different study localities (18% to 37.5%). Similar results were obtained in different parts of Zimbabwe (17.4 to 32.6%) (Pfukenyi et al., 2007), Al-Baha, Saudi Arabia (29.84 to 32.51%) (Ibrahim et al., 2015) and in Addis Aababa and DebreZeit, Ethiopia (57.2 to 76.4%) (Abebe et al., 2008). This geographical difference in distribution of positive cases could be explained by the management practices and the bio-security followed by the farm owners.

Analysis of risk factor with regard to the age of the calves revealed that there is no statistically significant association ( $\chi^2 = 3.363$ ,  $P = 0.186$ ) between the age of the calves and coccidian infection. This result agrees with the reports of Abebe et al. (2008), Tsegaye (2016), Alemayehu et al. (2013), Bekele et al. (2012) and Gillhuber et al. (2014). This similarity could be due to the fact that animal husbandry practices in the study areas are identical, and also, different age groups were kept and housed together without separation.

There was no statistically significant difference in prevalence of coccidian infection between male and female animals ( $\chi^2 = 0.437$ ,  $p = 0.508$ ), which is in agreement with the reports of Abebe et al. (2008), Heidari and Gharekhani (2014), Alemayehu et al. (2013) and Ibrahim (2016). This might be associated with the fact that different sex groups kept in similar husbandry system might have equal chance of accessing the oocysts. Despite this, previous studies done on adult cattle showed higher prevalence of *Eimeria* in female animals than in males (Manya et al., 2008; Rehman et al., 2011). Nevertheless, this could be attributed to the physiological stress loaded on female animals in relation to pregnancies and giving birth as compared to males (Radostits et al., 2007a).

The breed related prevalence of coccidian infection in the present study showed no statistical significant difference between breeds ( $\chi^2 = 0.103$ ,  $p = 0.748$ ), which is in agreement with the findings of Abebe et al. (2008) and Alemayehu et al. (2013).

The possible explanation for this similarity could be the fact that calf rearing condition in the study areas was identical and different breeds were housed together without separation. In contrast to the current findings, susceptibility differences were reported between local and cross breeds (Ibrahim, 2016). This breed susceptibility difference could be related to the dose of oocysts ingested and the species of *Eimeria* involved in

**Table 2.** Prevalence of calf coccidiosis in relation to various risk factors.

Risk factor		No. of sampled calves	No. of positive	Prevalence (%)	$\chi^2$	p-Value
Age (Months)	1-4	204	58	28.4	3.363	0.186
	5-8	102	28	27.5		
	9-12	78	14	17.9		
Sex	Female	208	57	27.4	0.437	0.508
	Male	176	43	24.4		
Breed	Local	124	31	25	0.103	0.748
	Cross	240	69	26.5		
Herd size	≤10 Animals	92	22	23.9	16.387	0.000
	11-20 Animals	117	46	39.3		
	>20 Animals	175	32	18.3		
Management	Semi intensive	296	79	26.7	0.281	0.596
	Extensive	88	21	23.9		
Body condition score	Good	171	20	11.7	51.447	0.000
	Moderate	164	50	30.5		
	Poor	49	30	61.2		
Fecal consistency	Normal	302	59	19.5	44.668	0.000
	Soft	67	28	41.8		
	Constipated	3	2	66.7		
	Diarrheic	12	11	91.7		
Barn hygiene	Good	164	24	14.6	29.393	0.000
	Moderate	191	59	30.9		
	Poor	29	17	58.6		

the infection (Taylor et al., 2007).

In the current study, coccidian infection was significantly higher in calves reared in large herd size than in small size ( $\chi^2 = 16.387$ ,  $P = 0.000$ ). This finding is in line with the report of Nasir et al. (2009). This similarity might be due to rapid spread of infection from calf to calf as well as greater contamination of feeding and watering troughs when animals are communally feed and overcrowded (Radostitis et al., 2007a). However, the current finding disagrees with that of Abebe et al. (2008). This variation might be due to the differences in the study seasons, husbandry practices and the treatment regime given to the calves.

There was no significant difference ( $\chi^2 = 0.281$ ,  $p = 0.596$ ) in the prevalence of coccidiosis and management systems. This result agrees with the report of Abebe et al. (2008), Alemayehu et al. (2013) and Temesgen (2016). This similarity might be due to equal chance of accessing the oocysts when grazing from contaminated field.

However, the current finding is in contrast with the previous report of Abisola (2004). This variation might be due to hygienic condition of the barn, nutritional status of the calves, contamination level of the feed, water, floor and treatment given to the animals.

There was statistically significant ( $\chi^2=44.668$ ,  $P=0.000$ ) difference in prevalence rate between fecal consistency and coccidian infection which agrees with the findings of Pundit (2009) and Alemayehu et al. (2013). However, this finding disagrees with the report of Abebe et al. (2008). In the present study, 91.7% diarrheic calves were found to be positive to *Eimeria*. However, there were no apparent clinical signs in most of the animals sampled for the study.

A strong significant association ( $\chi^2= 51.447$ ,  $P= 0.000$ ) was recorded between body condition score and coccidian infection in the current study. Similarly, Mehreteab et al. (2012) reported a higher infection rate in calves with poor body condition score than in calves with

good and moderate body condition score. This might be due to the weak immune status of the calves with poor body condition score. As a result, malnutrition and other parasitic infections result in immune compromised calves. This condition produces a higher infection rate in poor state animals than in good-state animals (Radostits et al., 2007b).

In the current study, association of *Eimeria* infection in relation to the hygienic status of calf house was verified ( $p < 0.05$ ). This result agrees with the report of Bekele et al. (2012) Mundt et al. (2005a, b) and Dawid et al. (2012). The similarity implies that poor sanitation in the calving and calf housing areas as well as poor management of housing favors infection of coccidiosis. Obviously, poor ventilation, droughts, poor calf nutrition, group pens, heavy stocking, cows present with calves, soiled bedding are regarded as risk factors for coccidiosis (Radostits et al., 2007b).

## CONCLUSION AND RECOMMENDATION

In conclusion, this study provides proof of a coccidian infection in dairy farms in Holeta and its environs. Hygiene of calf's house can be considered as a risk factor for the occurrence of coccidia. Large herd size, diarrheic faeces and poor body condition of the calves also increased the risk of infection with coccidia. However, sex, age, breed and management system of calves did not show any difference with the occurrence of protozoan parasite infection. In general, different risk factors were considered to affect the rate of infection of calves with this protozoan parasite.

Based on these findings, it was recommended that calves with severe diarrhea be isolated and treated with appropriate drugs; any possibility of fecal contamination of the farm and the calves be minimized. Further epidemiological investigations are required to determine the protozoan parasite species composition and their economic impact.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## REFERENCES

- Abebe R, Wossene A, Kumssa B (2008). Epidemiology of Eimeria infections in calves in Addis Ababa and DebreZeit Dairy Farms, Ethiopia. *Int. J. Appl. Res. Vet. Med.* 6(1):24-30.
- Abisola TO (2004). Studies on Bovine coccidian. [Apicomplexa: eimeriidae] In: Parts of Plateau State, Nigeria. Available at: <http://dspace.unijos.edu.ng/jspui/handle/123456789/129>
- Alemayehu A, Nuru M, Belina T (2013). Prevalence of bovine coccidia in Kombolcha district of South Wollo, Ethiopia. *J. Vet. Med. Anim. Health* 5(2):41-45.
- Bekele M, Ferid D, Yeshitila A (2012). Calf coccidiosis in selected dairy farms of Dire Dawa, Eastern Ethiopia. *Glob. Vet.* 9(4):460-464.
- Bhat SA, Juyal PD, Singla LD (2012). Prevalence of cryptosporidiosis in neonatal buffalo calves in Ludhiana district of Punjab, India. *Asian J. Anim. Vet. Adv.* 7(6):512-520.
- Brar APS, Sood NK, Kaur P, Singla LD, Sandhu BS, Gupta K, Narang D, Singh CK and Chandra M (2017). Periurban outbreaks of bovine calf scour in Northern India caused by *Cryptosporidium* in association with other enteropathogens. *Epidemiol. Infect.* 145(13):2717-2726.
- Central Statistical Agency (CSA) (2015). Federal Democratic Republic of Ethiopia: Central Statistical Agency Agricultural sample survey, Volume II, report on Livestock and Livestock characteristics, Bulletin No.578. Addis Ababa. pp. 12-15.
- Das M, Deka DK, Sarmah PC, Islam S, Sarma S (2015). Diversity of *Eimeria* spp. in dairy cattle of Guwahati, Assam, India. *Vet. World* 8(8):941-945.
- Dawid F, Amede Y, Bekele M (2012). Calf coccidiosis in selected dairy farms of Dire Dawa, Eastern Ethiopia. *Glob. Vet.* 9 (4):460-464.
- Debela E (2002). Epidemiology of gastro-intestinal helminthiasis of Rift Valley goats under traditional husbandry system in Adami Tulu district, Ethiopia. *Ethiop. J. Sci.* 25: 35-44.
- Dong H, Zhao Q, Han H, Jiang L, Zhu S, Li T, Kong C, Huang B (2012). Prevalence of coccidian infection in dairy cattle in shanghai, China. *J. Parasitol.* 98(5):963-966.
- Ernst JV, Stewart TB, Witlock DR (1987). Quantitative determination of coccidian oocysts in beef calves from the coastal plain area of Georgia (USA). *Vet. Parasitol.* 23(1-2):1-10.
- Gillhuber J, Rügamer D, Pfister K, Scheuerle MC (2014). Giardiasis and other enteropathogenic infections: A study on diarrhoeic calves in Southern Germany. *BMC Res. Notes* 7:112-120.
- Gupta SK, Singla LD (2012). Diagnostic trends in parasitic diseases of animals. In: *Veterinary Diagnostics: Current Trends*. Gupta RP, Garg SR, Nehra V and Lather D (Eds), Satish Serial Publishing House, Delhi. pp. 81-112.
- Heidari H, Gharekhani J (2014). Detection of *Eimeria* species in Iranian native cattle. *Int. J. Adv. Res.* 2(7):731-734.
- Heidari H, Sadeghi-Dehkordi Z, Moayedi R, Gharekhani J (2014). Occurrence and diversity of *Eimeria* species in cattle in Hamedan province, Iran. *Vet. Med.* 59(6):271-275.
- Hussin AG (2016). Prevalence and Associated Risk Factors of *Eimeria* spp. in cattle of Baghdad, Iraq. *J. Appl. Anim. Sci.* 9(1):37-44.
- Ibrahim DAYDN (2016). Prevalence and associated risk factors of calf coccidiosis in and around Asela Town, Southeast Ethiopia. *Prevalence* 6(3).
- Ibrahim MM, Soliman MF, Alghamdi AO (2015). Subclinical bovine coccidiosis in Al-Baha Area, Saudi Arabia. *Int. J. Vet. Sci. Res.* 1(1):023-028.
- Manya P, Sinha SR, Sinha S, Verma SB, Sharma SK, Mandal KG (2008). Prevalence of bovine coccidiosis at patna. *J. Vet. Parasitol.* 22:5-12.
- Mehreteab B, Ferid D, Yeshitila A (2012). Calf coccidiosis in selected dairy farms of Diredawa, eastern Ethiopia. *Glob. Vet.* 9:460-464.
- Mundt HC, Bangoura B, Rinke M, Rosenbruch M, Daugschies A (2005a). Pathology and treatment of *Eimeria* zuernii coccidiosis in calves: investigations in an infection model. *Parasitol. Int.* 54(4):223-230.
- Mundt HC, Bangoura B, Mengel H, Keidel J, Daugschies A (2005b). Control of clinical coccidiosis of calves due to *E. bovis* and *E. zuernii* with toltrazuril under field conditions. *Parasitol. Res.* 97:134-142.
- Nasir A, Avais M, Khan MS, Ahmad N (2009). Prevalence of *Cryptosporidium parvum* infection in Lahore (Pakistan) and its association with diarrhea in dairy calves. *Int. J. Agric. Biol.* 11(2):221-224.
- Pfukenyi DM, Willingham AL, Mukaratirwa S, Monrad J (2007). Epidemiological studies of parasitic gastrointestinal nematodes, cestodes and coccidia infections in cattle in the highveld and lowveld communal grazing areas of Zimbabwe. *Onderstepoort J. Vet. Res.* 74(2):129-142.
- Radostits OM, Gay CC, Blood DC, Hinchcliff KW (2007a). *Veterinary Medicine - A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*, 10th edition reviewed by Sameeh M. Abutarbush, BVSc, MVetSc, Diplomate ABVP, Diplomate ACVIM Saunders, USA Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2857440/>
- Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007b). *Diseases*

- associated with protozoa. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Goats, Pigs and Horses*. 10th ed. Philadelphia, Pennsylvania: Saunders Elsevier. pp. 1498-1515.
- Rehman TU, Khan MN, Sajid MS, Abbas RZ, Arshad M, Iqbal Z, Iqbal A (2011). Epidemiology of *Eimeria* and associated risk factors in cattle of district Toba Tek Singh, Pakistan. *Parasitol. Res.* 108(5):1171-1177.
- Sandhu BS, Singla LD (2005) Coccidiosis in bovines: an increasing problem. In: *Compendium of Winter School on Novel Approaches for Diagnosis and Control of Parasitic Diseases of Domestic and Wild Animals held from 07-27 October, 2005 at PAU, Ludhiana*. pp. 190-194.
- Taylor MA, Coop RL, Wall RL (2007). *Veterinary Parasitology*. 3rd ed. Oxford, UK: Blackwell Publishing. Available at: <http://trove.nla.gov.au/work/26713022>
- Temesgen GK (2016). Epidemiological studies on calf coccidiosis in dairy farms in South Wollo Zone Amhara Region, Ethiopia. *J. Vet. Sci. Technol.* 7(392):2.
- The Merck Veterinary Manual (2005). Merck & Co. Inc. and Merial Limited introduce the Ninth Edition of the Merck Veterinary Manual. Available at: <http://veterinarynews.dvm360.com/merck-merial-publish-2005-merck-veterinary-manual-0>
- Toaleb NI, El-Moghazy FM, Hassan SE (2011). Diagnosis of eimeriosis in cattle by ELISA using partially purified antigen. *World Appl. Sci. J.* 12:33-38.
- Thrusfield M (2005). *Veterinary Epidemiology*. 3rd Ed., Blackwell Science Ltd., Oxford, UK. pp.233-261.
- Tsegaye E (2016). Occurrence of coccidiosis in diarrheic calves in and around Asella town dairy farms. *World J. Biol. Med. Sci.* 3(3):48-54.
- Wadhawa A, Tanwar RK, Singla LD, Eda S, Kumar N and Kumar Y (2011). Prevalence of gastrointestinal helminths in cattle and buffaloes in Bikaner, Rajasthan, India. *Vet. World* 4(9):417-419.
- WoWAHA(2015). Wolmera Wereda Animal Health Agency. Statistical Abstract. Wolmera, FinfineLiyuZuria Zone, Oromia, Ethiopia.
- Yu SK, Gao M, Huang N, Jia YQ, Lin Q (2011). Prevalence of coccidian infection in cattle in Shaanxi province, Northwestern China. *J. Anim. Vet. Adv.* 10:2716-2719.

*Full Length Research Paper*

# **Determination of pH and water holding capacity of beef from selected butcher shops of Mekelle, Ethiopia**

**Hailelassie W/mariam Gebrehiwot\*, Endale Balcha, Yohanes Hagos and Kidane W/rkelul**

Department of Veterinary Medicine, College of Veterinary Medicine, Mekelle University, Mekelle, Ethiopia.

Received 13 March, 2018: Accepted 13 April, 2018

**A cross-sectional study was undertaken to determine the water holding capacity (WHC) of beef sold in selected butcher shops of Mekelle, Ethiopia and to establish the effects of pre-slaughter rests and age of the animals with pH and WHC of the meats. A total of 80 samples from 80 randomly selected butcher shops were sampled in 6 months study period of time, from May to October. Each sample was accompanied by questionnaires. The meats were taken to the laboratory to measure pH and water holding capacity using the filter paper press and drip loss methods. The means and standard deviations of filter paper press and drip loss were found as  $49.4 \pm 5.85$  and  $4.58 \pm 0.96$ , respectively. The age of the slaughtered animals had significant association ( $p < 0.05$ ) with the pH measured at 24 h post-slaughter, drip loss and filter paper press measurements. The pre-slaughter rest period had strong effect on pH ( $p < 0.05$ ) at 3 h post-slaughter. The present study concluded that water holding capacity and pH as quality indicators of meat are influenced by age and pre-slaughter rest of the slaughtered animals.**

**Key words:** Age, drip loss, filter paper press, pH, pre-slaughter stress.

## **INTRODUCTION**

Muscle is a tissue that consists predominantly of contractile cells of animals. It has different spatial arrangements of myofibrils. The spatial arrangement of the fibrillar network of muscle has great role in the quality of the meats (Bowker et al., 2014). The pH of muscle is approximated from 6.9 to 7.0. The variation in pH may come from physical exercise, nutritional deficiencies, age, sex and/or other factors of the animals (Zhang et al., 2010; Simela, 2005; Simela, 2004). Muscle is converted into meat during the processes of rigor mortis. In the pre rigor stage, the muscle is converted into the stage of rigor

and it becomes changed into meat. Fresh meat contains approximately 70 to 75% water, 15 to 20% protein, 5 to 10% lipids or fat and oils, 1 to 2% carbohydrates and 1% vitamins and minerals. At the rigor stage of meat, the amounts of these contents may be changed (Simela, 2005). Meat is said to be quality if it is suitable for use in specific products. The water holding capacity, the rate of decline in pH, flavor, juiciness, color, ethical quality and health quality are the quality indicators of meat. Good quality meat has a potential to have good water holding capacity, slow rate of declining pH, good appearance and

\*Corresponding author. E-mail: [whaile947@gmail.com](mailto:whaile947@gmail.com).

fatty smell and does not have zoonotic diseases (Aaslyng, 2002). The quality of meat can be altered either by pre-slaughter conditions of the animals or by post mortem conditions of the meat (Miller, 2007).

Water holding capacity (WHC) of meat is defined as the ability of meat to retain the water when external force is exerted. Such forces can be exhaustive starvation and thirst, prolonged traveling on foot, extreme heat or windy air condition of the environment when the animals stay, dry or humid storage of meat, and the orientation of cutting, grinding, or processing of meat (Miller, 2007). The majority of water in meat is held within the structures of the muscle itself. It can be found within the myofibrils, between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscles bundles (groups of muscle cells). Once muscle is harvested, the amount of water in meat can be changed depending on numerous factors related to the tissue itself and how the product is handled (Simela, 2005). Water held in the meat can have different names according to their location. Bound water is the water that is found within the protein of the meat. Entrapped water found within muscle structures. This water molecule may be held either by steric (space) effects and/or by attraction to the bound water but is not bound per se to protein. In early postmortem tissue, this water does not flow freely from the tissue, yet it can be removed by drying, and can be easily converted to ice during freezing. Entrapped or immobilized water is most affected by the rigor process and the conversion of muscle to meat. Upon alteration of muscle cell structure and lowering of the pH, this water can also eventually escape as wash out. This entrapped water is becoming free water when it is moved from the structure in pre rigor meat as a result of rigor mortis or post mortem effects. Free water is water whose flow from the tissue is unimpeded. Weak surface forces mainly hold this fraction of water in meat (Brondum et al., 2000). Maintaining as much of this water as possible in meat is the goal of meat handlers (Huff-Lonergan, 2005).

The pH level and the level of WHC of meat can be affected by the anti mortem events, such as lack of sufficient rest or extreme hot or extreme humid air condition of the lairage (pen), starvation and deprivation of water prior to slaughter of the animals for more than 12 h or the manner of handling like cutting of the meat during deboning and meat selling period of time, and while treating the meat to avoid meat spoilage can also affect the pH level and WHC of the meat (Lomiwes, 2008). This alteration of the pH level and WHC of the meat may come from the alteration of glycogen level of muscle of animals stressed during pre-slaughtered condition (Aaslyng, 2002; Miller, 2007). The stability of given meat can be ensured when the requirement of its water holding capacity is maintained and when there is a slow declining of its pH level. This means that the water holding capacity and the slowly declining of pH level of

that meat have zoonotic, economic and nutritional importance (Simela, 2005). Meat inspectors and local abattoir administrators of Mekelle city mentioned that the butchers brought their animals to slaughter house directly from the local markets without getting pre-slaughter rest (Mekelle, 2016). Those animals might have travelled from a longer distance in starved condition and stressed by moving here and there in the market and sent directly to the slaughter house without giving sufficient rest prior to slaughter. Besides, most of those animals are poor in body condition, progressively exhausted in work and older age. The above-mentioned professionals and other meat consumers also mentioned that the meat found in the butcher houses in Mekelle city looks dry, dark in color and not attractive to consume it. However, the problem is not yet studied. Hence, the present work will deal with the following specific objectives.

- (1) To determine the water holding capacity of beef sold in selected butcher shops of Mekelle
- (2) To establish the associations between age and pre-slaughter rest with pH and water holding capacity.

## **MATERIALS AND METHODS**

### **Study area**

The study was conducted in Mekelle city, Tigray region, Northern Ethiopia. Mekelle is an urban centre and the capital of Tigray region. The city covers an area of about 53 km<sup>2</sup>, with an estimated population of 215,546 people. It is located 783 km North of Addis Ababa (Figure 1). Currently so many regional, national and international people are visiting the city. From small to larger scales of restaurants are emerging in this city. It has 160 legal butcher houses that are distributed throughout the city and there are also some illegal butchers whose number is not determined yet (TRHDA, 2008).

### **Study design**

A cross-sectional study was employed for the present study.

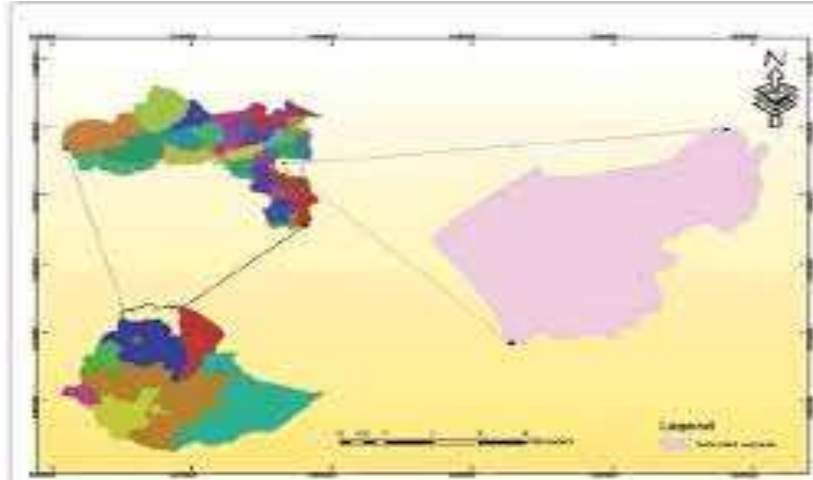
### **Sample size**

The sample was collected from Mekelle Municipality abattoir where the legal butcher shops send their animals for slaughter. For maximum precision, samples from 50% of the butcher shops, that is 80 samples were collected.

### **Sampling design and sample collection**

Simple random sampling technique was employed to select 80 butcher shops from the total of 160 (Mekelle, 2014) butcher houses. The longissimus dorsi (LD) muscle was taken because of its economic importance (cheapest in value in relative to the other parts of the muscle parts), long muscle and easy to sample (Preziuso and Russo, 2004). In order to know the age of the meat, all samples were taken from Mekelle Municipality abattoir. Collection of fresh meat sample was done immediately following





**Figure 1.** Location map of the study area.  
Source: Awetehegn (2016).

each slaughter by labeling each sample orderly in accordance to the code of the questionnaire. To prevent moisture loss during sample collection and preparation a tightly sealed plastic tube was used.

#### Questionnaire survey

Each sample was accompanied by questionnaire for characteristics of the owners' address, sex; characteristics of the slaughtered animal, the service provided, and observational characteristics of the meats.

#### Laboratory works

The work was conducted in the microbiology lab, college of Veterinary Medicine, Kalamino campus. The pH of the collected meat was measured using digital pH meter by probing it in to the meat for 30 to 60 s (Abraham and Kumar, 2000) and reading was recorded each sample along with its own code. Before taking the measurement it was calibrated using standard buffer solution (neutral) and it was rinsed with distilled water after taking each measurement. Firstly it was measured at 3 h period of time, and secondly, the same test was carried out at 24 h (Santos-Silva, 2001). The water holding capacity of meat was determined by two fundamentally different principles: Filter paper press method and the water drip loss method.

#### Filter paper press method

The external force used to drive out the water was the filter paper press method. Chromatography paper Whatman No. 40 was kept for 24 h in a dissociated 38% sulfuric acid in advance that it complies with 60% humidity and it helped to diffuse out the water freely through the paper (Kashif et al., 2014). Five grams of 24 h aged meat was homogenized on a metal plate. Out of this, 300 mg meat which was measured right after preparation was put on the Whatman paper No. 40 and then was placed between two slides on which a 100 g weight was placed on the top slide for 5 min so as to exert downward force and to release water from the meat as per the method described by Abraham and Kumar (2000).

The water released from the meat was wetting the paper and the boundary of that wetted area was demarcated using sharp pencil and was measured and reported in percentage of the ratio of the diameter of the meat to the diameter of the water wetted paper as per Mendiratta et al. (2008).

#### Water drip loss (Honikel bag method)

A 2.5 cm thickness slice, 2 × 5 cm width and length 24 h aged raw meat was cut parallel to the muscle fiber and hanged for 2 days in plastic sealed tube at 4°C as described by Brondum et al., (2000). The water drip out of the meat was measured and was reported in percentage of the ratio of weight of water loss to weight of meat prior to drip out the water (Diaz et al., 2010; Brondum et al., 2000; Honikel, 1998).

#### Data analysis

The qualitative and quantitative data were analyzed using simple descriptive and computer based statistical software (STATA 11) was used to analyze the data in order to compare means of age and pre-slaughter stress of the slaughtered animals on pH and water holding capacity of the meat. For the purpose of this study,  $p < 0.05$  was considered to be significant.

## RESULTS

#### Questionnaire survey

The characteristics of the slaughtered animals had shown that the majority (65%) of the slaughtered animals were old. Only 35% of the slaughtered animals were at the stage of bull. More than half (66%) of the respondents did not have lairage for giving rest for the purchased animals before they were slaughtered. 96.3% of the slaughtered animals were Zebu breed, only 3.7% of them were crossbred. More than 78% of the carcasses were dark in color and stored in the butcher shops for more than



**Table 1.** Owners' response for the characteristics of the animals slaughtered and their carcasses.

Characteristics		Number of respondent	Percentage
Age	Young/Bull	28	35
	Old/Steer	52	65
Rest	24 h rest	27	34
	Immediate slaughter	53	66
Breed	Zebu	77	96.3
	Cross	3	3.7
Age of carcass	12 h	17	21.3
	24 h	63	78.7
Color of meat	Dark red	63	78.7
	Light red	17	21.3

24h period of time before completely sold. Only 21.3% of them were light in color and sold before 24 h of storage time.

### Water holding capacity

The mean value of the drip loss of the total meat samples was shown as 4.58 with a low standard deviation (0.95). (Table 2). On the other hand, the filter paper press result was shown with the mean value of 49.4 with a relatively wider standard deviation (5.85) (Table 2).

### pH at 3 and 24 h post-slaughter

The mean pH value of the total sampled meat measured at 3 h post-slaughter was 6.2 with low standard deviation (0.26), and reduced to the mean value of 5.9 and standard deviation of 0.11 pH level measured at 24 h post-slaughter (Table 3).

### Effects of age on pH, drip loss, and filter paper press measurements

The age had statistically significant association with the pH measured at 24 h post-slaughter, drip loss and filter paper press ( $p < 0.05$ ) (Table 4).

### Association between pre-slaughter rest and pH at 3 h post-slaughter

There was statistically significant association between the pre-slaughter rest and the pH of the meats measured at 3 h post-slaughter ( $p < 0.05$ ) (Table 5).

## DISCUSSION

In the present study, it was found that 65% of the animals slaughtered were old whereas 35% were young (Table 1). This agrees with the report of Dawit et al. (2013) that 64.4% of the animals slaughtered at Mekelle abattoir were old in age. According to 78% of respondents, they spent more than 24 h to sell for a total carcass (Table 1). This prolonged storage of the meat might expose the meat to excessive drip loss. This is well supported by the reports of Walukonis et al. (2002). It was claimed that meat with low glycogen level will have dark color and high water holding capacity (Miller, 2007). According to the present study, the meat from 78.3% of butcher shops in Mekelle was dark red (Table 1). This disagreed with a similar study in Texas by Miller (2007) who found 2.3% of the sampled meats were dark red. The results of mean drip loss of meat of the present study on 97.3% Zebu and 3.7% crossbreds (Table 1) was relatively higher (4.58%) (Table 2) than the findings of Waritthitham et al. (2010) reported as 3.09% on Brahman breed which were fed with unknown type feeds; similarly, Yuksel et al. (2011) reported 1.8% drip loss on Holstein Friesian breeds fed on specific feeds. On the other hand, the result of filter paper press of this study was with the mean value of 49.4% (Table 2) of dark colored meat that was agreed with the reports of Gunenc's (2007) that had shown with mean value of 48.75 of PSE meat ( $p < 0.05$ ), and Miller (2007) also reported similar findings of dark colored meat with higher dark firm dried meat (DFD). These indicated that the low water holding capacity of the meat could cost butchers house significantly which was also supported by Huff-Lonergan (2005).

The pH at 3 h post-slaughter recorded was 6.1 to 6.6 (Table 3). This is comparable with the work of Troy (1999) who recorded 6.1 to 6.37. The higher pH might be

**Table 2.** Measurements of drip loss under gravity and filter paper press.

Parameter	Observation (n)	Mean $\pm$ SD
Drip loss	80	4.58 $\pm$ 0.95
Filter paper press	80	49.4 $\pm$ 5.85

**Table 3.** Measurements pH of the sampled meat at 3 and 24 h post slaughter.

Parameter	Observation (n)	Mean $\pm$ SD
pH at 3 h	80	6.2 $\pm$ 0.26
pH at 24 h	80	5.9 $\pm$ 0.11

**Table 4.** Effects of age on pH at 24 h and drip loss and filter paper press measurements.

Observation	pH at 24 h			Drip loss			Filter paper press		
	Mean	SD	P	Mean	SD	P	Mean	SD	P
Old/steer (52)	5.96	0.14		4.4	0.89		48.4	5.2	
Young/bull (28)	5.87	0.12	0.004	4.85	0.99	0.046	51.1	6.5	0.048

**Table 5.** Association between pre-slaughter rest of animals and pH at 3 h post-slaughter.

Rest category	Observation (n)	pH at 3 h post-slaughter		
		Mean	SD	P
24 h rest	27	6.37	0.27	0.0009
Immediately slaughtered	53	6.1	0.33	

due to pre-slaughter stress. Gunenc (2007) stated that pre-slaughter stress has effect on PM pH 3. The pre-slaughter rest of the animals had strong statistically significant ( $p < 0.05$ ) (Table 5), association with PM pH 3 of the meat which agrees with the statement of Lyczynski et al. (2006) that "pre-slaughter stress of animals had statistically significant effect on the early PM pH of meat ( $p < 0.05$ ). This is possibly due to the accumulation of lactic acid in muscle of slaughtered animals which was formed as a result of glycolysis process formed by pre-slaughtering stress of slaughtered animals and it is agreed with the reports of Zhang et al. (2010).

Water holding capacity and drip loss of the meat had statistically significant association with the age of the animals ( $p < 0.05$ ). This statement is supported by the findings of Kashif et al. (2014) who stated that water holding capacity and drip loss varied ( $p < 0.05$ ) (Table 4). between three groups of 1.5 and 2.5 years, and greater than 2.5 years olds animals. These findings also revealed that water holding capacity of meat was found lower in old age of the slaughtered animals. This might be because of higher accumulation of fat and lower moisture content in old age animals comparing to young age

animals which is in agreement with the suggestions of Zhang et al. (2010).

## CONCLUSION AND RECOMMENDATIONS

Determination of the water holding capacity (WHC) of meat is most important that it is one of the quality indicators of meat. The WHC of the meat was found as 49.4% by filter paper press results and water drip loss was 4.58%. These results were found as having statistically significant associations with age and pre-slaughter rest of the animals. Old age and lack of pre-slaughter rest of the animals resulted in increased ultimate pH level. The prolonged storage of that meat for more than 24 h lead to the spoilage of that meat due to high amount of water in the meat which is conducive for bacterial growth and that could be source of food born diseases and/or cause for the vigorous loss of the profit of the sellers.

The following possible recommendations were forwarded:

- (1) Owners of butcher shops should get training on pre-

slaughter handling of animals.

(2) Stressed animals should not be approved for slaughter.

(3) The study should be expanded to other breeds and species of animals.

## ACKNOWLEDGEMENTS

The authors are kindly indebted to thankful the College of Veterinary Medicine, Mekelle University that provided the laboratory and other materials to conduct the research work. They are additionally grateful for “Mekelle Zone Municipality abattoir service rendering unit” that cooperated to conduct the study.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Aaslyng M (2002). Quality indicators for raw meat. Danish Meat Research Institute, Roskilde. CRC Press LLC and Woodhead Publishing Ltd: pp. 1-13.
- Abraham RJ, Kumar KS. Practical manual For Meat Technology And Duality Control. Available at: <http://krishikosh.egranth.ac.in/bitstream/1/2040511/1/TNV-363.pdf>
- Awetehegn NB (2016). Precipitation and temperature trend analysis in Mekelle city, Northern Ethiopia, the Case of Illala Meteorological Station. Available at: <https://www.omicsonline.org/peer-reviewed/pprecipitation-and-temperature-trend-analysis-in-mekelle-city-northern-ethiopia-the-case-of-illala-meteorological-station-p-68927.html>
- Bowker B, Hawkins S, Zhuang H (2014). Measurement of water-holding capacity in raw and freeze-dried broiler breast meat with visible and near-infrared spectroscopy. *Poult. Sci.* 93(7):1834-1841.
- Brondum J, Munck L, Henckel P, Karlsson A, Tornberg E, Engelsen SB (2000). Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. *Meat Sci.* 55(2):177-185.
- Dawit G, Adem A, Simenew K, Tilahun Z (2013). Prevalence, cyst characterization and economic importance of bovine hydatidosis in Mekelle municipality abattoir, Northern Ethiopia. *J. Vet. Med. Anim. Health* 5(3):87-93.
- Diaz O, Rodríguez L, Torres A, Cobos A (2010). Chemical composition and physico-chemical properties of meat from capons as affected by breed and age. *Spanish J. Agric. Res.* 8(1):91-99.
- Gunenc A (2007). Evaluation of pork meat quality by using water holding capacity and vis-spectroscopy (Doctoral dissertation, McGill University).
- Honikel KO (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* 49(4):447-457.
- Huff-Lonergan E, Lonergan SM (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Sci.* 71(1):194-204.
- Kashif A, Shahzad A, Muhamed MK, Mohamed TK (2014). Effect of age on physico-chemical quality of buffalo meat. *Glob. Vet.* 13(1):28-32.
- Lomiwes D (2008). Rapid on-line glycogen measurement and prediction of ultimate pH in slaughter beef (Doctoral dissertation, Auckland University of Technology).
- Mozdziak P, Pietrzak I, Formanowicz D, Grzymisławski M, Wojciechowski J, Karamucki T (2006). Effect of environmental conditions on pork meat quality—a review. *Pol. J. Food Nutr. Sci.* 15(56):2.
- Mekelle (2016). Municipality abattoir service rendering unit, Mekelle Zone, Regional State of Tigray, Ethiopia.
- Mendiratta SK, Kondaiah N, Anjaneyulu AS, Sharma BD (2008). Comparisons of handling practices of culled sheep meat for production of mutton curry. *Asian Australasian J. Anim. Sci.* 21(5):738.
- Miller M (2007). Dark, firm and dry beef. Beef Facts-Product Enhancements. Available at: <https://fyi.uwex.edu/wbic/files/2011/04/Dark-Firm-and-Dry-Beef.pdf>
- Santos-Silva J (2001). The effect of weight on carcass and meat quality of Serra da Estrela and Merino Branco lambs fattened with dehydrated lucerne. *Anim. Res.* 50(4):289-298.
- Simela L (2005). Determination of meat quality, University of Pretoria, etd. Literature review: 4-44. Available at: <https://repository.up.ac.za/bitstream/handle/2263/29932/02chapter2.pdf?sequence=3>
- Simela L, Webb EC, Frylinck L (2004). Effect of sex, age, and pre-slaughter conditioning on pH, temperature, tenderness and colour of indigenous South African goats. *South Afr. J. Anim. Sci.* 34(5).
- Prezioso G, Russo C (2004). Meat quality traits of longissimus thoracis, semitendinosus and triceps brachii muscles from Chianina beef cattle slaughtered at two different ages. *Italian J. Anim. Sci.* 3(3):267-273.
- Tigray Region Housing development agency (TRHDA) (2008). Tigray Region Housing development agency, Housing report of 2007/2008.
- Troy J (1999). Biochemical and physiological indicators of beef, Dunsinea, Castleknock, dublin 15, Res. Rep. 13:4-34.
- Walukonis J, Morgan T, Gerrard E, Forrest C (2002). A technique for predicting water-holding capacity in early postmortem muscle. *Purdue swine Res. Rep.* 110-117.
- Wariththam A, Lambert C, Langholz HJ, Wicke M, Gauly M (2010). Muscle fiber characteristics and their relationship to water holding capacity of Longissimus dorsi muscle in Brahman and Charolais crossbred bulls. Available at: <https://bia.unibz.it/handle/10863/1018>
- Yuksel S, Turgut L, Yanar M, Aksu M, Kaban G, Unlu N (2011). Fatty acid composition, lean color and drip loss of the different muscles from young Holstein Friesian bulls finished on diets containing varied proportions of wet sugar beet pulp and wheat straw. *Bulgarian J. Agric. Sci.* 17(5):696-703.
- Zhang YY, Zan LS, Wang HB, Xin YP, Adoligbe CM, Ujan JA (2010). Effect of sex on meat quality characteristics of Qinchuan cattle. *Afr. J. Biotechnol.* 9(28):4504-4509.

**Related Journals:**

