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Case Report

Unilateral Surgical Amputation of Horn Due to Suppurative Sinusitis in Cow

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ABSTRACT

Dehorning or disbudding is the process of removing or stopping the growth of the horns and horn producing tissues after the horns have formed from the bud by different methods which can match to the size of the horn and age. The present case report is aimed to describe and document the surgical procedures, techniques of unilateral horn amputation and its outcome on six years old local breed cow that was referred to the veterinary teaching hospital (VTH), Addis Ababa University from nearby; Hiddi Veterinary Clinic. History stipulated as the cow was treated several times with antibiotics but didn't respond as the condition was getting worse. Based on the history and clinical findings, the case was diagnosed as suppurative frontal sinusitis. After aseptic preparation of the surgical site, stabilizing the animal and locally desensitizing the incision area; an elliptical skin incision with a distance of approximately (~1 cm wide) around the base of a right horn was performed for successful removal of the corium. Then, skin edge was opposed to assist the skin contraction by using the silk 2-0 size in cross mattress suture pattern. Then the area was properly bandaged with elastic bandages and properly secured to the normal horn and admitted home. Finally, with regular dressing, bandaging and lavaging of the dehorned site, the cow was successfully recovered after two months.

Keywords
Cow; Dehorning; Horn Injury.

INTRODUCTION

Horns are the pairs of hard, bonelike, permanent growth projecting from the head of cattle. They grow from a unique area of skin cells at the base of the horn. At about two months of age, horns become attached to the frontal bone of the skull.[1] A sinus lies within the skull beneath the horn bud. As the horn grows and attaches to the skull, this frontal sinus joins into the adjacent portion of the horn.[2] Dehorning or disbudding is the process of removing or stopping the growth of the horns and horn producing tissues after the horns have formed from the bud by different methods which can match to the size of the horn and the age of the animal for optimum effectiveness.[3] The principal reason for dehorning is to remove the risk of injury to other animals in the herd or to people working with the cattle. If the calves are dehorned at an early age, the nutrients will be utilized for growth of the animal. Introducing a horned animal in a polled herd is poor judgment and cows with horns often sell at reduced prices.[4]

The horns of cattle are the unique adaptations of the skin. The horn generating cells are located between the junction of horns and skin known as the corium, which is the site for horn growth.[5] Normally, horns begin as buds within the skin of the poll. At approximately 2-months of age, the horn buds become attached to the periosteum of the frontal bone overlying the frontal sinus. As the horns grow, the cornual diverticulum of the caudal portion of the frontal sinus extends into the most proximal portion of the horn.[6] The cornual nerve, a branch of the trigeminal nerve (cranial nerve V), provides sensation to the skin of the horn/horn bud region. Injection of a local anesthetic around the cornual nerve as it traverses the frontal crest and desensitizes the area.[7,8]

If dehorning is not properly done with the removal of the whole corium, then horns can start regrowing.[9] Similarly, im-

proper dehorning especially, at an older age, predisposes to frontal sinusitis in the calves.[10] Infection is a possible complication arising from dehorning, but this occurs mostly following invasive procedures that expose the sinus cavity to the external contaminated environment. Furthermore, the use of a surgical and a non-surgical instrument, including knives, Barnes dehorners, obstetrical wires, keystone (guillotine) dehorners, and saws may increase the risk of infection during dehorning.[11]

Normally, the wounds heal well with due care of post-operative treatment following dehorning. However, the procedure may cause several post-operative complications including bleeding, bacterial infections and fly contamination unless closely monitored. Operative animal requires 30-60-minutes for bleeding observation after dehorning but can be controlled through tourniquets, clamps or electric cauterizing. Not only these but also fly repellent should be provided for prevention of fly contamination, for 10-14-days.[12] Besides that, sinusitis is a complication of dehorning and an exclusive risk in older calves.[3] Sinusitis in cattle typically involves the frontal or maxillary sinus. Frontal sinusitis is generally associated with dehorning and maxillary sinusitis with infected teeth.[13] This case report is aimed to describe the surgical management of horn in cow due to frontal sinusitis as a sequel to the trial of dehorning by sharp material is described.

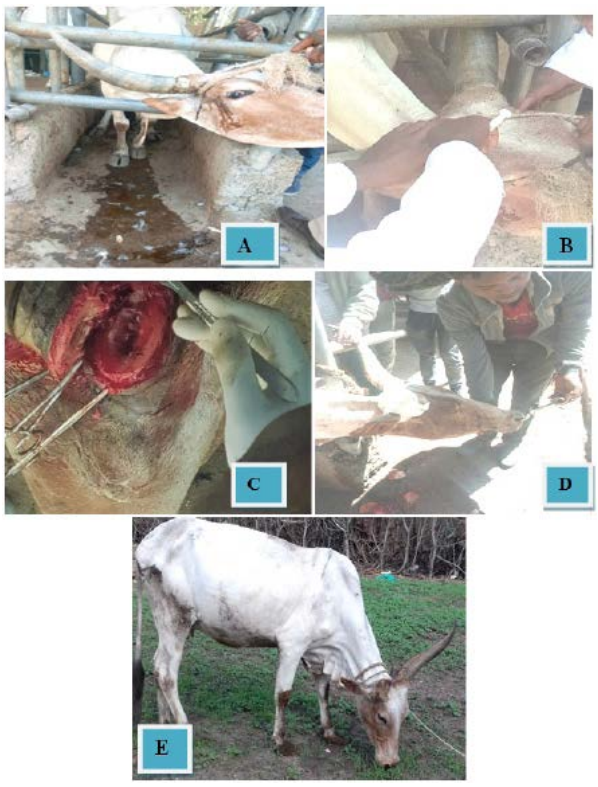
CASE REPORT

Case History and Clinical Examination

Six-years-old healthy local breed cow with a medium body condition was presented to Hidi veterinary clinic one month before and referred to the Veterinary Teaching Hospital (VTH), Addis Ababa University, Ethiopia. In the former veterinary clinic, the cow was treated with oxytetracycline injection (20 mg/kg) intramuscularly for three days at one-day interval but after one month, pus was oozing out of the wound without regressing. After two weeks, the owner understood that the wound did not heal properly and the dehorned site was septic and finally referred to VTH. The owner also complained of discharging of pus around the right horn following trauma around the base of the horn by sharp instrument (knife) for dehorning purpose at their home. When clinically examined, the right horn was partially sawed (cut) but the majority of the horn structure is intact and there was oozing of unpleasant discharge (serous to purulent) through the opening as shown in (Figure 1A).

The cow tries to mutilate her horn by rubbing against inanimate object frequently. The depth of the affected horn would measure approximately (3 cm in diameter×6 cm in depth) and was observed near the base of the right horn. There was swelling and pain with signs of vocalization on palpation of the wound area. Further close examination of vital organ parameters such as heart

Figure 1. *Dehorning and Its Surgical Procedure in Local Breed of Cow A) Presentation of Case B) Administration of Lidocaine to Block Cornual nerve C) Cutting the Injured Horn D) Putting Bandage on Dehorned Horn Base E) After Four Months of Surgical Treatment*



rate, respiratory rate, pulse rate and mucous membrane revealed within physiological limits. Finally, based on the history and clinical observation, the case was diagnosed as suppurative frontal sinusitis and decided to be managed surgically by unilateral horn amputation.

Pre-operative Preparation of the Cow

The cow was restrained adequately and the circumferential skin surface of the base of the right horn was prepared aseptically by washing with soap and water. Then the hair was shaved with blade and cleansed thoroughly with diluted Chlorhexidine solution. Finally, the area was scrubbed with iodine solution and dried before readying for dehorning.

Anesthesia and Animal Control

The cow was properly immobilized with the combination of physical and chemical method. Physically, the cow was handled with bull Handle assisted by personnel and kept in the well-built crush which adequately restrains the cow. Chemically, the cow was regionally anaesthetized with two percent lidocaine (2% lidocaine hydrochloride, jeil pharm. co. Ltd., Korea), loaded in a syringe with 18 gauge, 1-1.5 inch needle, 6 ml/side is injected halfway between the lateral canthus of the orbit on upper third and base of the horn just under the shaft of frontal crest to block the cornual nerve (Figure 1B) and waited for 5-minutes. In addition, the cow was sedated with diazepam (manufactured by Intas pharmaceutical Ltd., India) @ 0.1 mg/kg I.M.

Surgical Correction and Treatment

After well securing, managing and keeping the cow on appropriate direction, elliptical skin incisions with a distance of approximately (~1 cm wide) around the base of the right horn was done for successful removal of corium (Figure 1C). Then active bleeding was occluded by tamponade and ligation of major vessels in addition to splashing with adrenaline on bleeding site. Further, the skin was bluntly and gently detached from the bone by scissor and retracted rostrally to get the skin flap and make it easy for sawing the horn. Thence after adequately exposing the proximal part of the bone and horn base, the base of the horn below the corium was cut with dehorning saw and the subcutaneous tissues hanging the detached horn were cut by surgical and removed with the corium.

After that, the frontal sinus was exposed and detected for any gross pathological findings (Figure 1C). Upon holding and lowering of the dehorned horn part to one side and an intact one to upper side there was a little cascade of clear to serous discharge from the cavity. Then the cavity was washed with sterilized water and cleaned with gauze. Skin edges were partially opposed to assist the skin contraction by using the silk 2-0 size in horizontal interrupted mattress. The area was properly bandaged with elastic bandages as a second layer after well padding the opening with cotton sandwiched in between large gauze and soaking with weak iodine solution as a primary layer. The area was properly secured to the normal horn and admitted home (Figure 1D).

Post-operative Care and Outcome

Sporadic bleeding was noticed following the horn amputation but it is normal and helps to clean the wound unless continued for a long period. As there was a difficulty to oppose the skin flap completely following horn amputation, it was properly bandaged with elastic bandages and dressed regularly. The dressing of the wound was done at three, fifth, seventh days up to fourteen days until healed. Penicillin (24 mg/kg) and dihydrostreptomycin sulphate (30 mg/kg) (Pen & Strep® Norbrook, UK) was also administered I.M. for four days post-operation at Hidi veterinary clinic. Fly repellant was also done around the wound by using sterile vaseline. The owner was also advised to avoid leaving the cow in the yard after dehorning but advised to supplement good nutrition to facilitate wound healing. After two months, the wound was healed completely and at four months of follow-up the cow improved in body weight (Figure 1E).

DISCUSSION AND CONCLUSION

Dehorning is a delicate procedure that requires professional skill and expertise because poor technique and management of wounds may result in ample complications that will lead to septicemia and may leads to death of the animal in addition to delayed healing.[9] Several dehorning methods such as using chemical agents, including sodium hydroxide and calcium hydroxide are commonly used for the purpose of burning the corium.[4] Similarly, the electric hot-iron device is also used on calves up to twelve weeks of age. However, this method has certain drawbacks as it is also considered to be relatively painful. But in adult ones, it is not advisable rather using other methods such as dehorning wire and saw after administering appropriate local anesthesia and sedation.[13] This agrees with the current case management in terms of age and surgical management.

During dehorning, the removal of the skin corium by cutting with the detached horn is crucial unless it will re-grow again and promote infection in the unhealed wound.[11] This also agrees with present case where dehorning was performed in which the corium was properly removed and healed after few months. Some research also reported that the use of Barnes dehorners, knives, Burners, tubes, guillotine dehorners, saws and obstetrical wire, all may increase the risk of infection after dehorning.[10,14] In this case, the farmer used a knife to dehorn the cow and complications arose from the improper procedure, resulting in the development of frontal sinusitis.

Furthermore, the infected wound was not properly treated and managed except for the administration of systemic antibiotics. During dehorning, some behavioral signs observed include: head movement, tail wagging, tripping and rearing.[11] However, head rubbing, head shaking, extension of the neck, ear and tail flicking and reduced rumination are postoperative signs. Which agrees with the earlier signs reported by Sutherland et al.[15] In this case report, due to the infection of the wound, there was unpleasant discharges and pain with signs of reaction and reluctance to touch them as a result of the discomfort without prior administration of analgesic

drugs. This disagrees with similar case management by systemic analgesic (Flunixine meglumine) administration to reduce the pain and inflammation as reported previously.[13]

The post-operative management of dehorning is very important in preventing bacterial contamination of the wound. Dehorning has been shown to increase the incidence of developing the Bovine papillomavirus, Bovine leucosis virus and Tetanus. Pre or post use of non-steroidal analgesics has proven effective in reducing pain and swelling in the dehorned site.[12] In this case, no analgesic or suitable antibiotic medications were instituted by the owner, resulting in suppurative infection of the frontal sinus. However, proper wound management and medication was able to resolve the infection and ensured the healing process.

To sum up, surgical method of amputating horn is removal of the horn especially in adult animals at the base mainly below the corium when it is difficult to treat. But the procedure is highly invasive as the post-operative complications are high unless closely monitored and treated. In addition, the procedure is also highly complicated due to high probability of bleeding and delayed time of healing; predisposing the animal to infection of brain and myiasis. In this particular case report, the horn was surgically excised under aseptic condition and alleviation of pain through cornual nerve block. Finally, after removing the horn, the incision site was regularly dressed and bandaged and recovered after few months. So, for early recovery and positive outcome of the procedure, it should be managed early and regularly monitored for progression of healing.

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The figures used in the case report belong to the author's, captured in photograph while the author's were conducting the surgical procedure. The Addis Ababa University college of Veterinary Medicine and Agriculture ethical review committee had critically reviewed the proposal in the context of research ethics and conclude that, there was no ethical problem. The case report was conducted at a certain Veterinary Teaching Hospital without any additional fund and prepared as surgical case report for learning and teaching of professionals across the world especially for veterinarians with your cooperation for processing and publication.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Review

Non-Surgical Sterilization Methods in Male Animals: A Review

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ABSTRACT

Non-surgical sterilization technique in animals is an ancient practice and dates back to 7000 BC. Surgical castration in animals has been applied for centuries to control the animal population, advance genetic selection, improve calmness of aggressive animals and mainly to ensure and selectively provide high-quality meat production for human consumption. An ideal method of castration should cause permanent block to spermatogenesis and inhibit androgenetic enzymes with low-cost of treatment and doesn't affect the welfare of the animal. Even if operative methods are the main methods of castration, hormonal castration and chemical castration are also an alternative and equivalent method of sterilization. Over the past years, non-surgical sterilization has found application in male dogs, cats, monkeys, goats, bulls, hamsters and rabbits. Calcium chloride, lactic acid, sodium chloride, chlorhexidine, formalin, zinc tannate, zinc gluconate, glycerol, glucose, ethanol and silver nitrate are commonly used in chemical castration. After intratesticular application, degeneration of seminiferous tubules and Leydig cells, decrease in testosterone and sperm production, testicular atrophy is observed. In this review, the approaches of chemical castration were mentioned in different male animals.

Keywords
Male animal; Non-surgical sterilization; Castration; Chemicals; Testicle.

INTRODUCTION

Surgical castration is the most common method used to sterilize animals which are unsuitable for the genetic pool and also to eliminate masculine behavior.[1] Veterinarians are still practicing the open surgical method of castration which is the most effective and the only means of sterilization for male animals. Yet, castration by open surgery requires post-operative care to minimize the risk of hemorrhage and infection. Besides, this method has some disadvantages: it is not cost-effective and time-consuming with risk of severe post-surgical complications.[2,3]

In contrast to the surgical method, the challenge has been taken up by different reproductive biologists to develop a method of chemical sterilization, which may be a better alternative to surgical castration, as well as suited for mass-scale sterilization of male domestic animals without post-operative hazards.[2,4] In addition, over the last decades different chemical agents were tried to bring about castration using inorganic chemicals, immune-contraceptives and hormones including androgen,[5] progestogens,[6] androgens plus progestagens[7,8] and agonists for gonadotrophin releasing hormone (GnRH).[9]

Different researchers have evaluated non-surgical sterilization with injection of various hormones in many species of male animals, but these treatments failed to induce permanent sterility. Immunization techniques have also been used to induce antibodies against gonadotrophins and GnRH and had indicated that such immunization techniques vary in effectiveness and in duration of azoospermia. Adverse vaccination reactions were also observed as another disadvantage.[10,11]

Chemical sterilization has found application in some species of male animals such as monkeys, goats, bulls, hamsters, rabbits and dogs.[12,13] Open wound in surgical castration has always been an infection focus. Hence, researchers over the past years have also

tried various chemical agents such as danazol, glycerol, lactic acid, ferric chloride and ferrous sulphate, calcium chloride ($CaCl_2$), bacillus calmette-guerin (BCG), zinc gluconate (Neutersol) and 20% hypertonic saline solution for induction of chemosterilization.[14] However, all these chemical agents, following intratesticular injection had exhibited pain, pyrexia and even severe testicular inflammation (orchitis). Some agents like, cadmium chloride, glycerol, lactic acid had caused selective destruction of testicular tissue[15,16] with reversible testicular tissue damage.[17]

Even though the chemicals used had an effect on the destruction of testicular tissue, it had also complications and some drawbacks. For instance, in some cases, the interstitial portion of seminiferous tubules had regenerated after an initial phase of testicular atrophy and this had led to secondary male behavior causing management problems of the animals.[18] Due to such type of complications caused by the use of the aforementioned chemicals, an effective chemosterilizing agent is yet to be established.

An attempt has been made to induce sterilization by intratesticular cadmium chloride injection in male adult stray dogs[19] and scrub bulls,[20,21] but the mechanism of action of this chemical agent is still to be explored. A single bilateral intratesticular injection of calcium chloride had resulted in chemosterilization through the generation of free radicals as well as without induction of any general stress response in male Black Bengal goats.[13] As a result, chemosterilization is cheap, requires small number of staff and has positive effect on meat yield of castrated bulls. Moreover, it can also be used to control increasing population of stray animals such as dogs and monkeys.[14,20]

HISTORICAL BACKGROUND

History of sterilization of domestic animals dates back to 7000 BC with professional oversight of the practise being documented from the 15th to 19th centuries and it has been applied for centuries to control the number of animals, genetic selection, tranquillity of aggressive animals and most importantly, to ensure the production of high-quality meat for human needs.[22] Even if surgical castration has been considered a standard gold tool for sterilization of male animals, several drawbacks have been associated with this procedure such as high cost, time consumption, risk and need for post-operative care and management, small-scale application, requirement of a trained veterinarian and medical equipment.[3,20,23]

The main methods of castration are surgical castration, hormone inhibition, and chemical castration.[22] Hormonal castration not only directly affect the target organ but also indirectly affects and damage other organs. Whereas, chemical castration provides an ideal conditions of castration, economic expectations in consideration of the effect in a short time, the animal returns easily to normal physical activities after the process, a small number of person with less skill are required, so this method is more preferred than other methods of castration.[14,22]

Non-surgical chemical sterilization have better advantages over other methods and among these, reduction of pain and stress, elimination of hemorrhage, hernia, infection, myiasis and other surgical sequelae.[14,24] Accordingly, chemical castration has been suggested as a fast and low-cost alternative which could be used in a wide range of canine populations, especially in poor regions where problem is more intense.[25] *In situ* non-invasive approaches such as immune-castration, chemical castration and recently Eugenia caryophyllata essential oil castration minimize post-operative complications and costs associated with surgical castration.[23,26]

Accordingly, several attempts have been made in search of promising effective chemical agent, which included intratesticular injections (ITIs) of a various chemical agents to promote castration in various species such as Rhesus monkey (iron salt),[27] Rats (Hypertonic saline solution and Calcium chloride),[28-31] Canine (Calcium chloride, Zinc gluconate, Ethanol, Eugenia caryophyllata essential oil and Glycerol),[4,11,15,26,32-34] Feline (Calcium chloride, Zinc gluconate),[23,35,36] Bovine (hypertonic sodium chloride),[12,37,38] Donkey (Calcium chloride),[2] Ovine (Formalin)[21] and Caprine (Calcium chloride and chlorhexidine gluconate & cetrimide).[13,39]

Similarly, different researches are still being conducted and researchers have been interested in developing a method for chemical castration which might be a better alternative to surgical method.[14,20] Moreover, surgical method is not effective for large-scale application, especially for controlling large population size of undesirable mammals in the community like stray dogs. Besides this, post-operative care and management of the animal were also required to prevent infection.[13] In addition, vasectomies and vasal occlusion are less invasive surgical procedures than castration, still these procedures also carry similar anesthetic risks and post-surgical complications.[2,3]

An ideal chemical sterilizing agent should be permanent, low-cost treatment, effectively arrests spermatogenesis and androgenesis and not affecting the welfare of animals as well as without side effects.[4,14,40]

CHEMICAL STERILIZATION METHODS IN MALE ANIMALS

Control of fertility can be achieved through chemical contraceptives which prevents the birth of offspring but maintains fertility. However, by sterilization the animals renders infertile.[41,42] Contraception in domestic animals including dogs can be achieved through chemical reproductive control and immunological methods, which prevents pregnancy by sterilizing temporarily or permanently. These methods offers a humane and less expensive alternative to surgical sterilization.[34,43] Chemosterilization methods so far employed include hormonal methods, Immunocontraceptives and inorganic chemo-sterilants.

HORMONAL METHODS

Steroidal hormones such as progestin, estrogens and androgens administered *via* oral tablets or by parenteral injections have been used as reproductive inhibitors in owned dogs[44,45] and have the ability to temporarily control the reproduction of dogs. Gonad-

otrophin-releasing hormone is one of the target site for fertility inhibitors that control the release of the pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).[46]

GnRH agonists are also other hormonal methods and are proteins that act like GnRH and stimulate the production and release of FSH and LH. These methods can postpone puberty in both sexes and can also control the reproductive cycle.[45,47,48] Among GnRH agonists, deslorelin (Suprelorin®, Virbac, Pukete, Hamilton, New Zealand), is used for inhibition of reproduction in male dogs for at least a year in Australia and Europe.[46,49]

Azagly-nafarelin (Gonazon, Intervet International B.V., Amsterdam, The Netherlands) which is another GnRH agonist, has shown to decrease the concentration of testosterone for at least 6-months.[50] Gonazon is currently approved in the European Union but has not been yet brought into the European Union market.[46,51]

Immunocontraceptives

Immuno-contraceptive vaccines are another option and work by inducing antibody production against proteins or hormones essential for reproduction (e.g. gonadotrophins and GnRH) and therefore it can prevent pregnancy.[46,52] In contrast to GnRH agnostics, GnRH based vaccines target GnRH and prevent ovulation and spermatogenesis. Zona pellucida (ZP)-based vaccines inhibit egg-sperm binding and fertilization. These vaccines are commonly used for population control in wildlife species and domestic dog populations.[51,53]

GonaCon™ is a GnRH-based vaccine which had been registered as a contraceptive for white-tailed deer, horses and feral donkeys in the United States that induce infertility for at least 1-6 years after a single injection.[46,54,55] In contrast to ZP-based vaccines, GonaCon™ has not yet been proven effective in dogs since early formulation of GonaCon™ showed abscesses and draining at the injection site after the injection.[52]

A new formulation of GonaCon™ has been produced since then and tested in an experimental study carried out on captive dogs in Mexico.[52,56] This new formulation of GonaCon™ did not result in abscesses at the injection sites. According to Kisiel's research finding, it had been indicated that immunization techniques vary in effectiveness and in duration of time to cause azoospermia. Adverse vaccination reactions were also observed as another disadvantage of this method.[46,48]

Inorganic Chemosterilants

An ideal inorganic chemosterilizing agent would be one that effectively arrests spermatogenesis and androgenesis as well as libido with absence of toxic or other side effects. Inorganic chemo-sterilants are simple, easy with less technical ability and cost effective for mass-scale sterilization.[2,20,34]

The low cost, ease of use and cultural acceptance of a sterilization method that does not require removal of the testes make inorganic chemo-sterilants a valuable tool for large-scale sterilization campaigns, particularly in areas lacking clinical facilities or skilled staff.[25,41,46,57] Thus, chemical castration could be an attractive option for developing countries with limited resources for surgical management programs.[58]

Researchers over the past five decades have also tried various inorganic chemosterilizing agents such as cadmium chloride,[16] ferric chloride and ferrous sulphate,[27] Danazol,[59] BCG,[60] glycerol[61] and lactic acid[18] for chemical castration by intra testicular injection in laboratory and domestic animals. After intratesticular injection, all these agents exhibited pain, pyrexia and even severe orchitis.[20]

Chemo-sterilizing agents such as cadmium chloride, glycerol and lactic acid caused selective destruction of testicular tissue with reversible testicular tissue damage.[15] As result, in some cases, the interstitial portion regenerated after an initial phase of testicular atrophy and this led to secondary male behavior, which caused management problems of the animals Due to the above complications caused by the use of aforementioned chemicals, an effective chemosterilizing agent is yet to be established.[18,34]

For the past 10-years, an attempt has been made to induce sterilization by intratesticular injection of calcium chloride ($CaCl_2$),[2-4,12,30,62] Zinc gluconate (Neutersol)[22,63] and 20% hypertonic saline solution[64,65] in bull, cat, dog, donkey, goat and albino mice. As indicated above, different chemical agents were tried and used over the past years. Accordingly, sodium chloride, calcium chloride and zinc gluconate were the most commonly used by different researchers and thus research findings regarding the aforementioned chemicals are described as follows.

Sodium chloride solution: Hypertonic saline is a solution easy to administer, easily available and inexpensive. In a study, 20% hypertonic saline solution was injected bilaterally into the rat testes at different areas with a total amount of about 0.5 to 1 ml in each testis and reported that the coagulation necrosis was observed in all testes.[22,34,64]

Kwak et al[65] revealed that severe degenerative changes were seen in testicular seminiferous tubules and massive infiltration of inflammatory immune cells in animals injected with hypertonic saline solution. Additionally, researchers indicated that intratesticular hypertonic saline injection seems to be an alternative method to orchiectomy and surgical castration. However, further analysis and laboratory work would be required to ascertain the potential utility of this approach in dogs.[28,64,65]

In another study, it was suggested that intratesticular injection of 20% hypertonic saline could be an effective method for non-surgical sterilization of the young male dogs but not adult dogs.[22,34] Intratesticular injection of 20% hypertonic saline solution induces coagulative necrosis of leydig cells and seminiferous tubules as well as extensive testicular fibrosis. Based on the observed lesions, to arrest the testicular development and testoster-

one synthesis to cause sterility, the intratesticular injection should be performed during the first 20-days of life of the calves. Thus, intratesticular injection sodium chloride solution can be used as a viable alternative to surgical orchiectomy in calves.[37]

The use of inorganic chemo-sterilants in male dogs is an attractive option because it removes the disadvantages such as costs of surgical sterilization and post-operative care.[45] Furthermore, in countries like Romania and Bahamas where surgical castration of male dogs is not culturally accepted, chemical castration offers a reasonable alternative.[58,66,67]

Calcium chloride ($CaCl_2$): Non-surgical male sterilization techniques have been evaluated as a means to avoid the potential health complications, expense, expertise and facilities required for surgical sterilization procedures. Calcium chloride is one of the most promising chemical agent which has been utilized to chemically castrate a variety of species since 1978.[68] Different species of experimental animals such as rats, dogs, cats, cattle, donkey and buck were used to evaluate the efficacy of calcium chloride as chemosterilizing agent.[14,20]

Calcium chloride causes necrosis, fibrosis and degeneration of seminiferous tubules and Leydig cells following intratesticular injection. As a result, there was reduction in production of spermatozoa, testosterone and sperm counts in a dose-dependent manner. Although calcium chloride did not affect animals' food intake, it changes blood parameters such as blood cortisol due to stress and swelling of the testicles persisted for three weeks following injection and the behavior of the animals returned to normal a month after treatment.[2,3] More studies are ongoing to standardize and validate formulation, dosage, and administration protocol for calcium chloride.[44,46]

Intratesticular injection of calcium chloride has been applied to a variety of animal species including mice,[3,29] dogs,[3,4,11] cats,[36] goats,[13,39] bulls[12,19,68] and donkey,[2] in a variety of formulations and concentrations.[36]

A single bilateral intratesticular injection of calcium chloride was found to be effective, economical, easy to perform and does not require removal of testis in male cats and dogs. It had caused permanent sterilization and it is free from pain, chronic stress and had been found a simple alternative method to surgical castration.[3,36]

One bilateral intratesticular injection of 20% calcium chloride in ethyl alcohol solution was optimum for rendering dogs azoospermic and lower testosterone levels by 70% without side effects.[11] After single intratesticular injection of calcium chloride, destruction in seminiferous tubules and leydig cells, decrease in testosterone and sperm production, atrophy in testicles were observed. Thus, calcium chloride was effective and can be used as substitution of surgical castration in bull, buck, rat, cat and dog.[3,12-14,29,30,36,38,69]

Furthermore, calcium chloride injection has major role on impairing male reproductive organs resulting in sterility together with various key reproductive parameters including testicular histology but other general parameters were intact.[69]

Procedure of intratesticular injection of calcium chloride solutions: each intratesticular injection can be administered using a sterile 21-gauge needle directed from the caudo-ventral aspect of each testis approximately one cm from the epididymal tail and towards the dorso-cranial aspect of that testis, so that the solution can be deposited over the entire route by linear infiltration while withdrawing the needle from the proximal end to the distal end. All animals should be restrained through a gentle handling and proper care. The intratesticular injections should be given very carefully in order to prevent seepage of the solution from the injection site and avoid any other intramuscular injections. First, the animals can also be restrained through the proper handling procedure in a prior experiment in the case of vaccination and blood collection, etc. The animals should be kept under routine clinical observations and follow-up (Figure 1).[3,14,22]

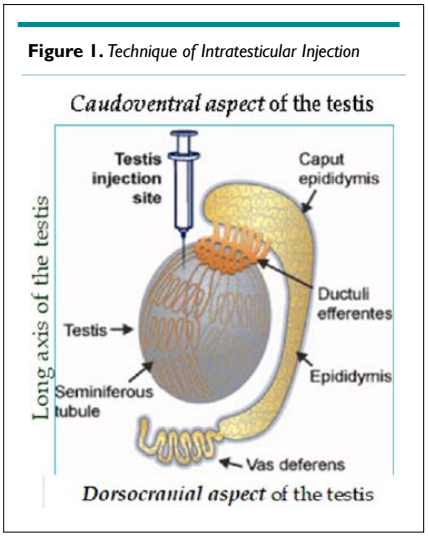

Figure 1. *Technique of Intratesticular Injection*

Zinc gluconate neutralized by arginine (Neutersol): Zinc gluconate was the first chemical sterilizing product to fulfil the criteria three key criteria of an ideal method of chemical sterilization. That is, first, effectiveness and large-scale application in male animals. Second, high margin of safety without adverse effects for the environment. Thirdly, it has to be permanent and irreversible following a single treatment. The first product obviously fulfilling these criteria was zinc gluconate. It was first described by Fahim et al[70] who injected Neutersol (Pet Healthcare International, Inc., Columbia, MO, USA), a Zinc Gluconate-based chemical sterilant into the epididymis of dogs. It is a zinc-gluconate solution neutralized by arginine to a pH of 7. Subsequently, Neutersol was injected into the testes of puppies and in the testes of adult dogs.[32,71]

Zinc gluconate is currently available in Mexico, Colombia, Bolivia, and Panama as Esterilsol and in the United States as Zeuterin (both through Ark Sciences, New York). The cost of Esterisol is about US$15 per dog (medium size).[44,46] The procedure involves injecting a predetermined amount of zinc solution based

on scrotal width into each testis of puppies 3-10-months of age.[41] Histological findings of the testis after injection of Neutersol at the age of 2.5-months revealed almost complete fibrosis of the seminiferous tubules and Leydig cells.[41,72]

Zinc gluconate neutralized by arginine (Neutersol, Addison Biological Laboratory Inc., Fayette, Missouri, USA) was approved in 2003 by the US Food and Drug Administration for chemical sterilization of male puppies. Injected into the testicles, this chemical causes sclerosis of the testes and sterility. Neutersol induced sterilization in 99.6% of the 223 male puppies aged between 3 to 10-months.[73] During intratesticular injection of the chemical, sedation is recommended to prevent movements of the dog. Correct injection technique was found critical for the safe use of Neutersol® in order to avoid ulceration of the scrotum and painful swelling of the testes.[41,71]

Unlike Neutersol®, Esterilsol® is currently used as chemical sterilizing agent in Mexico. However, both products still can't produce a long-lasting decrease in testosterone level that can significantly reduce nuisance and aggressive behavior. Studies using these models of male contraception report no or minimal signs of discomfort have been observed following injection, but a transient increase in testicular diameter may follow the injection, resulting in scrotal swelling. Additional local and systemic reactions reported after intra-testicular injections include scrotal ulceration and dermatitis, scrotal self-mutilation, preputial swelling, vomiting, diarrhea, anorexia, lethargy and leukocytosis. Also, unlike surgical castration, this kind of chemical sterilization does not eliminate gonadal sources of testosterone.[36,71,72]

In 2010, Esterilsol received regulatory approval for use in dogs three months and older in Bolivia, Colombia and Panama after introduced by Ark Sciences as Esterilsol in Mexico in 2008. In Colombia, it is also approved for use in cats.[46,57]

In a study carried out in the Galapagos, Ecuador, severe injection-site reactions occurred in 3.9% of the 103 dogs treated with zinc gluconate. Initially, the basal testosterone concentration in treated dogs decreased but two years after treatment. The effect was similar to untreated dogs.[44,57] Thus, secondary male characteristics such as roaming, marking, aggression and mounting may be displayed.[46,50]

A study was carried out with Esterilsol in Mexico and found that this compound induced azoospermia or aspermia in 52 out of 53 dogs when administered a single dose per testis.[74] The incidence of ulcers in dogs after poor injection technique was higher (2.6%) and incidence had decreased after proper injection technique using new needles for each injection. A similar study conducted in Brazil for dogs had concluded that zinc gluconate could be regarded as a permanent sterilant with no observed sign of behavioral alterations or severe discomfort following intratesticular injection.[25]

Another study in Isabela Island, zinc gluconate has got greater social and cultural acceptance as an option for surgical method since this technique provides low cost, ease of use, does not require removal of the testes, large-scale use, particularly in remote locations lacking sophisticated clinical facilities or skilled surgeons and staff.[57]

Esterilsol is administered *via* an injection to each testicle with either a 28 gauge, 3/4 inch or a 30 gauge, 1/2 inch needle, specified by manufacturer depending on the dose determined for the individual dog. Esterilsol is labeled for use in dogs with an individual testicular width of 10-27 mm. Ark Sciences has recommended light sedation is enough to ensure that the dog holds still during the injection (General anesthesia is not necessary; so, reversible sedation is commonly used so that dogs are awake and alert in as little as 15-20-minutes after the Esterilsol injection/zinc neutering).[44]

Experienced practitioners report that the process of measuring the testicular width (to determine dose), preparing the injections and administering the injection into each testicle takes two to five minutes. Following intratesticular injection, proper follow-up is critical to reduce the risk of injection site reactions. Thus, this formulation was found to cause permanent sterility in 99.6% of treated dogs.[14,44,46] Further investigation is needed to identify risk factors for adverse reactions to zinc gluconate and to develop strategies for its avoidance.

CONCLUSION AND RECOMMENDATIONS

In general, non-surgical methods of sterilizations have greater safety over operative method since each chemical substance has minimal side effects. Besides, it can be preferred for its less post-operative complication, cheap, small number of staff requirements, ease of application and especially positive effect on meat yield in bulls and pigs. In conclusion, non-surgical sterilizations approach and techniques are significant in terms of both animal welfare and cost effectiveness especially in male animals.

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Review

Medicinal Value of *Croton macrostachyus* and *Solanum incanum* against Causative Agent of Foodborne Diseases

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ABSTRACT

Foodborne diseases are a public health threat which causes a large economic impact across the worldwide. *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella species* (*S. species*), *Staphylococcus aureus* (*S. aureus*) and many more other organisms are the leading causes of foodborne illness and death in the world. Increment of antibiotic resistance exhibited by the actions of microbial infectious agents has led to screening of several medicinal plants for their potential antimicrobial activity. Therefore, the aim of this paper is to review on the medicinal value of *Croton macrostachyus* (*C. macrostachyus*) and *Solanum incanum* (*S. incanum*) against causative agents of foodborne disease. Antimicrobial compounds of medicinal plants differ from antibiotics as they have fewer side effects, better patient tolerance, relatively less expensive, acceptance due to a long history of use and being renewable in nature. There are so many medicinal plants used to treat foodborne diseases which associated with gastroenteritis in humans and animals, among plants *S. incanum* and *C. macrostachyus* are the common for treatment of foodborne diseases associated with diarrhea. *S. incanum* has different bioactive substances which have medicinal importance against skin diseases, abdominal pains, fever, stomachaches and indigestion, treatment of dandruff, wounds, sore throat, angina, ear inflammation, liver disorders, wart and ringworm and treatment of cow driosis, dermatophilosis, foot rot, pasteurellosis, black leg, fasciolosis and snake bite. Bioactive compounds which present in *S. incanum* are including alkaloids, steroids, saponins, tannins, glycosides, flavonoid and terpenoids. *C. macrostachyus* is medicinal plant which have bioactive compounds including terpenoids, alkaloids, flavonoids, lignoids, proanthcyanidins, sesquiterpenoids and lupeol, saponnins, resins, crotepeoxide. *C. macrostachyus* have medicinal value in treatment of malaria, rabies, gonorrhea, wound, diarrhea, hepatitis, jaundice, abdominal pain, cancer, toothache, pneumonia, typhoidand gastrointestinal disorder. Which is also used as abortifacient and uterotonic to expel retained placenta. Therefore, *S. incanum* and *C. macrostachyus* have different medicinal value against foodborne disease and drug-resistance infectious agents. However, an advanced study have not been conducted on the extract of pure bioactive compounds and toxicity analysis. Therefore, further studies should have to be conducted to extract pure compounds from these medicinal plants for pharmaceutics industry.

Keywords
Bioactive substance; Drug resistance; Food borne disease; Infectious agent; Medicinal plants.

INTRODUCTION

Foodborne diseases are a public health threat which causes a large economic impact across the worldwide. It can be caused by pathogens including bacteria, viruses and parasitic organisms.[1] Foodborne pathogens including *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*), *S. species*, *Staphylococcus aureus* (*S. aureus*) and many more other organisms are the leading causes of foodborne illness and death in the world.[2] Foodborne zoonotic diseases often occur due to the consumption of contaminated food-stuffs especially from animal products such as meat and milk.[3,4]

Diarrheagenic *Escherichia coli* (DEC) strains is one of the causative agent of foodborne diseases which leading causes of diarrheal illnesses throughout the world both in humans and animals.[5,6] Gastroenteritis due to foodborne disease is one of the most common illnesses in Ethiopia, and it is a leading cause of death among people of all ages in the country.[7] The occurrence of *E. coli* in foods of animal origin in Ethiopia is arguably high due to many reasons like illegal slaughtering of animals in open fields, un-

hygienic slaughter practices in the abattoirs, and the risk of disease is high because of a widespread tradition of raw meat consumption.[8,9] DEC contamination is usually associated with contaminated water and food, their presence reflects fecal contamination of both human and animal's origin.[10]

S. aureus is an opportunistic foodborne gram positive pathogen which causes many human and animal diseases.[11] *S. aureus* causes an infection in animals and humans which including inflammations of bone, meningitis, septicemia and rashes, mastitis (inflammation of mammary gland in bovine) and inflammation of lower part of the foot in poultry.[12,13] *S. aureus* is an important pathogen both in community acquired and healthcare associated infections due to its fast growing resistance to antibiotics. In particular, methicillin resistant *S. aureus* presents major infection control problems and threats globally.[14]

Antimicrobial resistance in bacterial pathogens is a worldwide challenge associated with high morbidity and mortality. Multidrug-resistant patterns in gram-positive and negative bacteria have resulted in difficult to treat with conventional antimicrobials. Broad spectrum antibiotics are liberally and mostly unnecessarily used and result in emergence of resistance bacteria.[15] The emergence of resistant infections caused by most bacteria has led to mortality and morbidity and there is an urgent need to find solutions to combat bacterial resistance.[16] The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections; this is due to excessive use of antimicrobial, incorrect antimicrobial dosage and unregulated access to drugs.[17] The reservoir of resistant bacteria in food animals implies a potential risk for transfer of resistant bacteria, or resistance genes, from food animals to humans.[18,19]

The increasing antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.[20] Medicinal plant has great role in care of primary health of humans and animals due to its biological and medicinal activities, high safety margins and ability to overcome drug resistance action of pathogens.[21,22] Medicinal plants are an important source of traditional drugs, modern medicines, folk medicines, nutraceuticals, pharmaceutical intermediates and entities for synthetic drugs since plant extracts contain many medicinal metabolites such as alkaloids, glycosides, terpenoids, flavonoids and lignins.[23] Antimicrobial compounds of medicinal plants differ from antibiotics as they have fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature.[24] Ethnoveterinary practice to animal health care is as old as the domestication of various livestock species.[25] There are so many medicinal plants used to treat foodborne diseases which associated with gastroenteritis in humans and animals, among plants *Solanum incanum* (*S. incanum*) and *Croton macrostachyus* (*C. macrostachyus*) are the common for treatment of foodborne diseases associated with diarrhea.[26]

C. macrostachyus have different phytochemical which act as antidiarrheal, antimicrobial, anticonvulsant and sedative, anthelmintic, antidiabetic, anti-inflammatory, antileishmanial, antioxidant, antiplasmodial, larvicidal and antifungal activity. The secondary bioactive compounds which have pharmaceutical activities are including alkaloids, amino acids, anthraquinones, carbohydrates, cardiac glycosides, coumarins, essential oil, fatty acids, flavonoids, phenolic compounds, phlobatannins, polyphenols, phytosteroides, saponins, sterols, tannins, terpenoids, unsaturated sterol, vitamin C, and withanoides.[27-29] The bioactive compound tannin is found in *S. incanum* have antiseptic and vasoconstrictor affects, and also have the ability to decrease diarrhea *via* forming protective layers of the mucous membranes. But, not only tannin, and also astringent phenolic compounds, triterpenoids and saponins also have an antidiarrheal effect.[30]

Fruit extracts of *S. incanum* exhibited potent antibacterial effect while leaf extracts showed antimicrobial activity against the *E. coli*, *S. pyogenes*, *S. aureus*, and *P. aeruginosa*. Therefore, the main objective of this paper is to review on the medicinal value of *C. macrostachyus* and *S. incanum* against causative agents of foodborne diseases.

MEDICINAL PLANTS AND PATHOGENIC BACTERIA

Study of Medicinal Plant

Medicinal plants are the sources of bioactive compounds which used mainly for medicinal purposes to tackling different diseases of animals and humans especially in developing countries.[31] The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogen.[32,33] Medicinal plants have been widely used all over the world and formed the integral part of basic health care in many countries including Ethiopia. Modern veterinary medicine are not well-developed in most of the countries and it is estimated that the traditional remedies are sometimes the only source of therapeutics for human population and animal.[34]

Ethiopia is well-known with having tremendous medicinal plants which has been used for treatment of livestock and human ailments, but medicinal values of plant is not well-documented which impedes widespread use, evaluation and validation. In recent time, many young people are lack of indigenous knowledge of medicinal plant, this raised from limitation of transferring indigenous knowledge of this medicinal plants.[35,36] Application practices of medicinal plant range from administration of the roots, barks, stems, leaves and seeds to the use of extracts, infusions, powders and decoction from the plant.[37,38] The innovational approach between plants and healthy is launching new generation of multi-component of botanic drugs, dietary supplements and plant produced recombinant proteins.[31,39]

Ethnoveterinary medicine is the scientific term for traditional animal health care, encompasses the knowledge, skills, methods, practices, and beliefs about animal health care found among the members of a community. The knowledge base differs not only from region to region but also among and within communities. It has been developed through trial and error and deliberate

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experimentation.[40]

The plant-based human and livestock health care persists and remains as the main alternative treatment for different ailments in Ethiopia, largely due to shortage of pharmaceutical products, prohibitive distance of the health service stations, unaffordable prices by small holder farmers and pastoralists for conventional drugs, emergence and re-emergence of certain diseases and appearance of drug resistant microbes and/or helminthes.[41] Studying of medicinal plant have great role in innovating the novel drug or alternative antimicrobial components from different plant parts. World Health Organization (WHO) estimates show that about 80% of African people rely on folklore herbal medicine for their primary health care due to scarcity or exorbitant prices of modern medicines. It is believed that herbal medicines exhibit less toxic and cost effective that the synthetic counterpart modern one.[42] Research investigation of *S. incanum* and *C. macrostachyus* have been showed the medicinal value of these plants which including in treatment of gastrointestinal infections, toothaches, dermatitis, wound, malaria and dandruff.[43]

***Solanum incanum*:** *S. incanum* have different names in different languages such as; Hiddii loonii (Afan Oromo), Embouy (Amharic) and Sodom/bitter apple (English)) as indicated in Figure 1(B). The name *S. incanum* is derived from Latin words, 'Solamen' meaning "relief" indicating the narcotic effects of the plant and 'incanum' meaning "white".[44] It is a native African shrub which belongs to Solanaceae family and also grows in many regions of Africa, Middle East and Far East Asia.[45-47] *S. incanum* is a delicate perennial plant often cultivated as an annual crop. It grows 1-3 m high with simple leaves, ovate, elliptic, 2.5-12 cm long and 2.5-8 cm wide. The fruit is fleshy, less than 3 cm in diameter on wild plants but much larger in cultivated forms. Botanically the fruit is classified as a berry and contains numerous small, soft seeds which are edible, but are bitter because they contain an insignificant amount of nicotinoid alkaloids. Flowers are in clusters along the branches corolla pale to deep blue, purple, occasionally white. Fruit is spherical, green, often striped or mottled with white, turning yellow to orange brown when ripe. *S. incanum* is the bushy herbal plant, native to north and north eastern Africa including Ethiopia. It found at forest edges and in bush land, grass land from sea level-up to 2500 m altitude.[48]

Figure 1. *Medicinal Plant, A: Croton Macrostachyus and B: Solanum Incanum (Taken by: Dr. Tagesu Abdisa)*

Phytochemical constituent of *S. incanum*: The bioactive substances which exist in fruit of *S. incanum* includes: alkaloids, steroids, cardiac glycosides, saponins, flavonoids, tannins, oxalates and cyanogenic glycosides, but anthraquinones is absent.[49] The leaves of *S. incanum* contains alkaloid, steroid, glycosides, flavonoid, saponin, tannins, triterpenes and cardiac glycosides. Root of *S. incanum* consist of spirostanol saponin, four known saponins, indioside D, dioscin, protodioscin, methylrotodioscin and steroid glycoalkaloid solamargine. Aerial part (the part above soil) consists of two steroidal glycosidal alkaloids, solasonine and solamarginine and non-steroidal components like three phenylalkanoic acids, benzyl-O-b-d-xylopyranosy-b-D-glucopyranoside, flavonoids, chlorogenic acid, adenosine and new compound kaempferol.[50-52]

The medicinal value of *S. incanum*: *S. incanum* is a medicinal plant widely employed around the world as antifungal, antiulcerogenic, antinociceptive, antipyrectic, anti-spasmolytic, orexic, hypoglycemic, antimicrobial, antischistosomal, laxative, antimicrobial, hemorrhoids and snake bites.[48,53] *S. incanum* have various medicinal importance, it was employed in eastern and southern Africa for the treatment of skin diseases, general infections, abdominal pains, fever, stomachaches and indigestion, treatment of dandruff (fruit), wounds, sore throat, angina, ear inflammation, liver disorders, wart, ringworm.[54,55] In Oromia region the fruit of *S. incanum* is the main medicinal plant used for the treatment of cowdriosis, dermatophilosis skin lesion, foot rot and pasteurellosis and also the root decoction of *S. incanum* is used to treat black legs, fasciolosis and snake bite.[56]

As one research reported[57] that *S. incanum* have the antimicrobial activities against gram positive and gram negative bacteria such as: *S. aureus*, *Bacillus subtilis*, *P. aeruginosa*, *E. coli*, *Salmonella paratyphi* and *Vibrio cholera*. Fruit of *S. incanum* is used for control of tick infestation in Ethiopia.[58] *S. incanum* also used in pain relieve in toothache and cure poison of snake bites as well as used in curdling milk or making cheese, leather tanning and soap making due to its alkaloid constituent.[59,60]

The extraction of *S. incanum* have great role in treatment of cancer, which containing the active ingredient solamargine, can induce apoptosis *via* up regulation of tumor necrosis factor expression and activation of the mitochondrial apoptosis pathway, and also have therapeutic effect in treatment of patient with actinic keratosis.[61,62] The alkaloid also rapidly induced membrane blebbing which could not be prevented by chelating either the intracellular or extracellular calcium ions though it was inhibited by some polyethylene glycols. It also disrupted the cytoplasmic actin and microtubules.[63]

The *S. incanum* have antitypanosomal compound which is steroidal alkaloids derivatives such as cilistol-A, solasonine, solamargine and chaconine; this compounds act as antitypanosomal activity against *Typanosoma cruzi* and *Typanosoma brucei* and also have anti-leishmaniasis, *S. incanum* antiprotozoal effect has been reported for the first time against *P. falciparum*, *L. infantum*, *T. brucei* and *T. cruzi*.[64,65]

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***C. macrostachyus*:** *C. macrostachyus* is commonly known as; rush foil (English), Bakkaniisa (Afan Oromo) and Bisana (Amharic) as indicated in Figure 1(A). It belongs to the Euphorbiacea with 300 genera and 8,000- 10,000 species and most abundant plant in the tropics.[66,67] It is native to Ethiopia, Eretria, Kenya, Tanzania, Uganda and Nigeria which is a medium-sized deciduous tree of East Africa particularly wide spread between 200-2500. In mountainous forests and savannah of the tropical regions and ever green bush land areas that receive between 700-701, 200 mm rainfall annually.[68-70] The name of Croton comes from a Greek word 'Kroton' which means ticks, because of the seeds' resemblance to ticks, the specific name "macrostachyus" is a contraction of two words, the Greek word "macro" meaning large and "stachyus" relating to the spike, hence, a species characterized by large spikes.[71] *C. macrostachyus* is regarded as a multipurpose tree by subsistence farmers in Ethiopia, Kenya, and Tanzania and the species has potential in playing an important role in the primary healthcare. The bark, fruits, leaves, roots, and seeds of *C. macrostachyus* are reported to possess diverse medicinal properties and *C. macrostachyus* is used as herbal medicine for at least 61 human and 20 animal diseases and ailments. In the distribution area there is a high degree of medicinal use consensus for bleeding, blood clotting, cancer, constipation, diarrhea, epilepsy, malaria, pneumonia, purgative, ringworm, skin diseases or infections, stomach ache, typhoid, worm expulsion, and wounds.[29,72]

Phytochemical constituent of *C. macrostachyus*: The genus Croton is rich in terpenoids (diterpenoids and triterpenoids), alkaloids, flavonoids, lignoids, proanthcyanidins and volatile oils containing monoterpenoids, sesquiterpenoids and some shikimate-derived compounds. Previous studies showed the existence of crotin (a chalcone), lupeol (a triperpene), crotepoxide (a cyclohexane diepoxide), proteins, fatty acids, saponins, resins and alkaloids.[29,73] The activity of *C. macrostachyus* stem bark extracts is comparable to studies where antiplasmodial activity has been related to a range of several classes of secondary plant metabolites including alkaloids and sesquiterpenes, triterpenes, flavonoids, inonoids, and quassinoids.[73]

Medicinal value of *C. macrostachyus*: Traditionally *C. macrostachyus* used for treatment of malaria, rabies, gonorrhea, wound, diarrhea, hepatitis, jaundice, scabies, toothache, abdominal pain, cancer, typhoid, pneumonia and gastrointestinal disorders and as ethnoveterinary medicine.[74-76] Pharmacological studies on *C. macrostachyus* indicate that it has a wide range of pharmacological activities such as anthelmintic, antibacterial, antimycobacterial, antidiarrheal, antifungal, anticonvulsant and sedative, antidiabetic, anti-inflammatory, antileishmanial, antioxidant, antiplasmodial, and larvicidal effects.[29] The leaves and shoots of *C. macrostachyus* are used to treat fever and oedema and also mashed leaves used for treatment of hemorrhoids. Moreover, the maceration of *C. macrostachyus* stem bark is used as abortifacient and uterotonic to expel retained placenta.[77]

C. macrostachyus have the activities of against diarrhea; traditional healers in Ethiopia use a wide range of medicinal plants with antidiarrheal properties.[28] The chemical constituent in the *C. macrostachyus*, Terpenoids such as abietic acid and steroids like phytosterols have been showed to inhibit production of prostaglandin E2 which have a crucial role in stimulation of intestinal secretion, therefore it has antidiarrheal activities.[78,79]

Study of Pathogenic Bacteria

***Escherichia coli* and its infection:** *Eschericia coli* a gram negative non-spore forming facultative anaerobic rod. Genus *E. coli* belongs to the bacterial group formally called "coliforms" which are member of the "enterics" known as Enterobacteriaceae family.[80] The strain of *E. coli* are motile because they have flagella arranged in peritrichous, but those lack of flagella are non-motile.[81] *E. coli* is a catalase positive, oxidase negative, lactose fermenter, coccobacillus gram negative non spore forming rod shaped bacteria.[82] *E. coli* requires the ability to adapt to variations or extreme changes in temperature, pH, and osmolarity conditions commonly encountered in nature. For example, the exopolysaccharide (EPS) production of *E. coli* is associated with heat and acid tolerance, and the alteration of lipid composition in membranes is induced by heat stress.[83] *E. coli* can survive for a long time in water, especially at cold temperatures. Water trough sediments contaminated with bovine feces can serve as a long-term (>8-months) reservoir of *E. coli*, and the surviving bacteria in contaminated troughs is a source of infection.[84]

E. coli are one of the major etiological agents of calf diarrhea with severe lethal outcome and major damage to the livestock industry worldwide, consequently highly mortality rate in calves under three weeks old and up to 3-months-old has been reported.[85,86] Diarrhea in animals especially in young cattle is one of the frequently encountered clinical syndromes, it causes economic losses from mortality and morbidity, treatment costs and time spent on care as well as chronic ill-thrift nature of calf diarrhea.[87]

DEC can be divided into six pathogens includes; Enterotoxigenic *E. coli* (ETEC), Eenteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHE), Enteroinvesive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC) and Vero or shiga like toxin producing *E. coli*.[88,89] The food types most commonly associated with outbreaks of food poisoning due to *E. coli* are mostly of bovine origin, in particular, beef and beef burgers and unpasteurized milk.[90,91] However, it has been increasingly recognized that fresh vegetables and fruits other than beef or beef-product can be the sources of Shiga toxin-producing *E. coli* (STEC) infection.[92-94]

DEC is the most common pathogenic to human which cause bloody diarrhea and HUS or TTP but, it has no any clinical disease except diarrhea in cattle and other animals.[95,96] The human can be infected with *E. coli* through consumption of contaminated food of bovine origins, fecal contaminated of food products and direct contact with infected animals.[97] *E. coli* are part of the intestinal microflora of health animals and humans which can carry genes that allow them to produce toxins known as Vero toxins or Shiga-like toxins. Verotoxigenic *E. coli* (VTEC) are not pathogenic to ruminants, but they cause serious diseases in humans worldwide, including diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome, and sometimes death.[98] Shiga toxin producing *E. coli* is

ubiquitous food and water borne pathogens inhabiting different animals, wildlife and humans.[99-101]

Shiga toxin producing *E. coli* are associated with dysentery in calves. As it was reported that, *E. coli* O157:H7 was isolated from fecal samples of calves.[102] The pathogenic *E. coli* is carried in the intestinal tract and excreted in the feces, and also present on the skin which can be transferred to carcass during evisceration. The contaminate during slaughter may cause the spread of *E. coli* to carcass and the human can be infected after consumption.[103]

After ingestion of *E. coli*, the bacteria bind to intestinal mucosa and begin releasing Shiga toxin. The produced toxins disrupt protein synthesis in the epithelial cells lining intestinal mucosa, small vessels of the intestine, kidney and brain resulting in thrombotic microangiopathy. *E. coli* attaches to the microvilli of intestinal epithelial cells and initiates colonization and establishes intimate attachment which is responsible for the translocation of a variety of effectors which alter the structure and function of host cells.[104] Vero toxin producing *E. coli* are widespread in animals but ruminants thought to be the natural reservoir. *E. coli* colonizes the terminal colon of cattle and can be shed in very large numbers by herd mates known as "super shedders". Feces containing these organisms act as a source of contamination for a variety of foods and the environment.[105]

***Staphylococcus aureus* and its infection:** *S. aureus* is a facultative anaerobic which belongs to the family of Staphylococcoceae. It is gram positive coagulase and catalase positive and non-spore forming non-motile which have paired cocci in grape like bunch structural shape.[106] *S. aureus* causes mastitis in milking herds, and occasionally purulent dermatitis in their milkers.[107] *S. aureus* is the most commonly affected part of the body due to infection is the skin. The skin infections are including small benign boils, folliculate, impetigo, cellulitis and invasive soft tissue infections.[108]

S. aureus is an important cause of hospital and community acquires infections which frequently resistance to many different classes of antimicrobial drugs. It was first described as hospital acquired methicillin-resistant *S. aureus* (MRSA) in 1961 as nosocomial infections. Later on the pathogen was observed in healthy humans without hospitalization and it terms community acquired MRSA.[109,110] Livestock-associated MRSA was first associated with human disease in 2003, when a MRSA clone associated with a reservoir in pigs and cattle was isolated from a human.[111] MRSA has been found in horse and livestock. MRSA can be transmitted from livestock to care takers, during milking and treating of animals.[112-114] *S. aureus* is unique in its ability to clot plasma during coagulase test which distinguish Staphylococcus epidemidicus and S. hyicus (coagulase negative). *S. aureus* are tolerant to high concentration of salt and show resistance to heat. *S. aureus* is grown on mannitol salt agar with golden orange to yellowish colonies which distinguish it from *S. epidermidis* and *S. hyicus*.[115,116]

Antimicrobial Resistance

Antimicrobial resistance is one of the most common disastrous factor to global public health. Bacterial resistance to antibiotics has increased rapidly within recent years which have led to the increase in the incidence of infectious diseases caused by those multi-drug resistant bacteria. Infections caused by multi-drug resistant bacteria involve higher morbidity, mortality, and a burden to health care systems. The common cause for antimicrobial resistance effect of bacteria is drug residue, due to contamination of meat products with antibiotic residues when the human consume meat with drug residue, then the appearance of resistant bacteria may occur.[117-119] The main mechanisms of microorganism for antimicrobial resistance are antibiotic inactivation, target modification, changes in permeability and altering metabolic pathway, decreased antibiotic penetration, β lactamase production and efflux pumps.[120]

The antimicrobial drug resistance can be caused by intrinsic and acquired methods. Intrinsic mechanisms are those specified by naturally occurring genes found on the bacterial chromosome. Intrinsic mechanism is due to the presence of outer cell membrane in gram negative bacteria and expression of efflux pumps. The acquired resistance mechanism is due to chromosomal mutation and horizontal transfer of mobile genetic elements from other bacteria in the environment *via* carrying the resistance gene including plasmids, transposon and integrons. The genetic element can be transferred from one bacterium to other through conjunction (cell-to-cell contact between elements), transduction (bacteriophage facilitated transfer of genetic information) and transformation (uptake of free deoxyribonucleic acid (DNA) from the environment.[71,121-123] Gram negative bacteria possesses high permeability barrier for numerous antibiotic molecules. Their periplasmic space contains enzymes which are capable of breaking down foreign molecules, so gram negatives are less susceptible to plant extraction than gram positive bacteria.[124]

Drug Extraction and Phytochemical Bioactive Compounds

Crude extraction of medicinal plant: The plant extract drugs are new interest as antiseptics and antimicrobial agents in medicine which have safer biologically active compounds with acceptable therapeutic index for development of novel drugs.[125,126] Plant extracts have more active target sites against drug resistant pathogens.[127] Plant extraction is the procedure of the separation of medicinal active portions of plant from inactive component part of plants, which undergoes by using solvents diffuse into the solid plant material and solubilize compounds with similar polarity.[128] Plant extraction can be carried out in different steps including collection of plant parts, drying, size reduction (grinding into pieces), extraction by mixing with solvents, filtration, concentration by rota vapor, drying and reconstitution. The quality and quantity of crude extraction may be influenced by several factors including plant part, solvent, procedure and ration of solvent to plant.[129,130]

The concept of solubility 'like dissolve like' which mean polar solvent (water, ethanol, methanol) extracts out polar substances and non-polar solvents extracts out non-polar solvents (petroleum ether, acetone and chloroform), this mechanism is depending of functional groups of solvents.[131] There are so many methods for crude extraction from plant including, maceration,

soxhlet apparatus, infusion. Maceration is one of the extraction techniques which have been used in wine making and medicinal plant research investigation. It involves soaking plant materials in a container with solvent and allowed to stand at room temperature for 3-7-days with frequent agitation.[129]

Phytochemical secondary bioactive compounds: Plant produces many secondary metabolites which constitute an important source of antimicrobial, pesticides and pharmaceutical drugs and also medicinal plants are best source to obtain novel drugs.[132,133] Phytochemicals can be classified as primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds.[134] Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. Alkaloids are used as anaesthetic agents and are found in medicinal plants.[135]

Glycosides moieties including saponins, cardiac glycosides and flavonoids are used to inhibit tumor growth, serve to protect against gastrointestinal infection which caused by pathogens those that cause enteric infection.[136,137] Secondary metabolites of medicinal plants have different mechanism of actions against bacteria agents. The mechanism can be through inhibition of bacterial enzymes, affecting cell division, bacterial membrane disruption and affecting virulence genes. The mode action of alkaloid is through inhibition of FtsZ (Flamenting temperature sensitive mutant Z) assembly and its GTPase activities which causes cell elongation without cell division. Protein FtsZ have greater role in bacterial cell division which have homolog of the eukaryotic tubulin, then this protein has the affinity to bind with alkaloid which inhibit cell division.[138]

The hydrophobic nature of essential oils (non-polar bio active compounds) disrupted metabolic activities and energy production line of the bacterial cells. It affected on the plasma membrane which makes bacterial cells more permeable to other bioactive compounds.[139] The mechanism of phytochemical secondary metabolites act with forming complexes with bacterial cell and inhibit cell activities. Flavonoids can for complexes with bacterial cell proteins and interfere with cell activities in the process of bacterial adhesion. Tannin and flavonoids have an antibacterial effect which can bind with proteins and inhibiting cell protein synthesis.[140]

CONCLUSION AND RECOMMENDATIONS

Foodborne diseases and drug resistance infectious agents are the most common disaster against the health of animals and humans throughout the world. Bacterial resistance to antibiotics has increased rapidly which have led to increase in the incidence of infectious diseases caused by those multi-drug resistant bacteria. Infections caused by multi-drug resistant bacteria involve higher morbidity, mortality, and a burden to health care systems. Moreover, foodborne disease which caused by *E.coli* and *S. aureus* is the most common in developing countries especially in young animals and children. However, medicinal plants are the natural resources which have so many bioactive secondary metabolites against pathogenic organisms. Bioactive compounds have different mechanisms against infectious agents through affecting cell division, enzyme inhibition of bacteria, bacterial membrane disruption, affecting virulence genes and disruption of protein synthesis of bacteria. Therefore, *S. incanaum* and *C. macrostachyus* have different medicinal value against foodborne disease and drug resistance infectious agents. These medicinal plants have various novel bioactive compounds, although further research studies have not been conducted on the extract of pure bioactive compounds and toxicity analysis. Therefore, the further study should have to be conducted to extract pure compounds rather than crude extract from these medicinal plants for pharmaceutics industry.

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Review

Probiotics and Its Potential Role in Poultry Production: A Review

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ABSTRACT

Probiotics are live microbial feed supplements, which have an effect on the host animal by means of enhancing its intestinal microbial balance. A probiotic is a culture of a single bacterial strain, or a mixture of different strains, with the aim of eliminating the colonization of pathogens in the gastrointestinal tract of poultry. The main sources of probiotics in poultry are strains of microorganisms such as Lactobacillus, Enterococcus and Bacillus and fermented dairy products like yoghurt, cultured buttermilk and cheese. A good probiotic is characterized by its ability to exert a beneficial effect on a host, resistance to low pH and bile salts, adhere and colonizing of the intestinal epithelium, non-pathogenic to host and produces antimicrobial substances towards pathogens. It also boosts immune responses, improves the growth performance and productivity of poultry and increases the quality of meat and egg. Thus, probiotics are considered to fill the gap in the poultry industry due to diseases and antimicrobial resistance of pathogenic bacteria as well as environmental conditions that cause serious problems and economic losses in many countries. With current consumer preferences tending toward purchasing products from livestock grown without antibiotics and feed additive, the ingredients in this review paper presented the beneficial applications probiotic may have in poultry production.

Keywords
Antimicrobials; Microorganisms; Poultry production; Probiotics.

INTRODUCTION

Poultry production has become an important part of economic activity in many countries. In large-scale intensive production, poultry production is exposed to many stressful conditions and diseases that result in serious economic losses. Currently, prevention measures using antimicrobial agents have been questioned due to the evolution of antimicrobial resistance among pathogenic bacteria. Accordingly, probiotics are being considered as the best option to fill the gap and already used by some farmers in preference to antibiotics.[1,2]

Probiotics were first coined by Lilly and Stillwell in 1965 and derived from the Greek word, meaning 'for life' and in contrast to antibiotic, probiotics defined as "substances secreted by a microorganism that stimulates the growth of another". Later in 1989, the definition was modified by Fuller as "live microbial cultures which beneficially affect the host by improving its intestinal microbial balance".[2,3]

At the end of 20th century, the concept of probiotics evolved from a hypothesis first proposed through the Russian scientist and Nobel Laureate, Elie Metchnikoff, who cautioned that the lengthy, healthy existence of Bulgarian peasants; resulted from their consumption of fermented milk products. He believed that consumption of the fermenting Lactobacillus positively influenced the microflora of the gut, decreasing the toxic microbial activity of the pathogenic bacteria population.[4,5]

A probiotic also referred to as direct-fed microbial, is a culture of a single bacterial strain, or a mixture of different strains, that can be fed to an animal to improve its health. A variety of different types of bacteria, and in some cases even undefined cultures, have been tested as probiotics in poultry. The aim of many studies involving direct-fed microbials has been to exclude the colonization of pathogens in the gastrointestinal tract of poultry.[6-8]

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Probiotics can prevent pathogen colonization of the gut and reduce the incidence or relieve the signs and symptoms of numerous diseases due to dysregulated immune responses. Probiotics seem to function by influencing both intestinal epithelial and immune cells of the gut, but the details of these effects are still being unraveled. So, probiotics enhance the host immune system and used to prevent diseases. The beneficial effects of probiotics can vary between strains so, the selection of the most suitable ones will be crucial for their use in the prevention or treatment of specific diseases. In order for a potential probiotic strain to exert to its beneficial effect, probiotics need to be delivered to the desired sites in an active and viable form. The viability and activity of probiotics in the products have been frequently cited as a prerequisite for achieving numerous beneficial health benefits.[9,10]

SOURCES OF PROBIOTICS

Microorganisms Used as Probiotics

The success of probiotics depends upon the survival and stability of the probiotics, the strain, the age, host specificity of the strain, dose rate, physiological and nutritional status of the bird genetic make-up of the host.[11] In contrast to the crop, proventriculus, and gizzard, the small intestine contains a large number of facultative anaerobes such as Lactobacillus, Streptococci, and anaerobes like Bacteroides and Bifidobacterium species. Probiotics colonize three different regions within the gastrointestinal tract (GIT); enterocyte, cecal and colonic epithelium.[3,12,13]

Lactobacillus, Bifidobacterium, Enterococcus are among the most commonly used Genera of probiotic microorganisms in human nutrition. whereas yeast especially *Saccharomyces cerevisiae* plays a major role in ruminants; while Bacillus, Enterococcus and Lactobacillus are more likely to be efficient in pigs and poultry.[14,15] Some of the important strains of microorganisms considered as probiotics are listed in Table1.

Table 1. *Strains of Microorganisms Frequently Used as Probiotics in Poultry*

Genera of Probiotic Microorganisms	Strain of Microorganisms
Lactobacillus species	*L. acidophilus, L. casei, L. crispatus, L. gasseri, L. fermentum, L. johnsonii, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus, L. bulgaricus*
Bifidobacterium species	*Bifidobacterium bifidum, B. breve, B. lactis, B. longum, B. infantis, B. adolescentis, B. animalis*
Enterococcus species and Lactic acid bacteria	*Enterococcus faecalis, E. faecium, Lactococcus lactis, Leuconostoc mesenteroides, Pediococcus acidilactici, Streptococcus thermophilus*
Non-lactic acid bacteria	*Bacillus cereus, Bacillus subtilis, Escherichia coli* strain nissle, *Propionibacterium freudenreichii, Aspergillus oryzae, Saccharomyces acidophilum, Saccharomyces boulardii, Saccharomyces cerevisiae*

Source:[16,17]

Other Sources of Probiotics

Yoghurt and other fermented milk products such as cultured buttermilk and cheese are among dominant foods used as sources of probiotics that provide a relatively low pH environment that facilitates the survival of the probiotic bacteria.[18] Lactic acid bacteria, Bifidobacteria and other microorganisms isolated from fermented milk products. Spontaneous milk fermentation has a long history in different regions of Mongolia and Africa, and the use of beneficial microorganisms in fermented dairy products has been practiced for many generations. These traditional fermented milks contain complex compositions of Lactic acid bacteria species and therefore provides a useful source of probiotic strains.[19]

In addition, probiotics can also be found in non-dairy fermented substrates such as soy-based products, cereals, legumes, cabbage, maize, pearl millet and sorghum.[16,18] The other sources of probiotics include breast milk, the human gastrointestinal tract, and the guts of several animal species, including pigs, rats, and even poultry. Recent researches were performed to assess traditional fermented products for their potential capacity as natural resources of probiotic bacteria. Generally, most of the microorganisms isolated from fermented products belong to the Lactobacillus genus.[20-22]

PRINCIPLES FOR SELECTION OF PROBIOTICS IN THE POULTRY INDUSTRY

During the selection of probiotics strain, safety accesses must be kept in mind regarding production relating to the technological aspects, application, survival, and colonization in the host and their health benefits.[23]

Resistance to Low pH and Bile Salts

Acid tolerance is one of the general criteria that is considered during the selection of potential probiotic strains to secure their viability and feasibility.[24] The survival of ingested probiotics in different parts of the gastrointestinal tract varies with the strain. Some strains are rapidly killed in the stomach while others, such as strains of Bifidobacteria or *Lactobacillus acidophilus*, can pass through the whole gut at very high concentrations. Numerous *in vitro* and *in vivo* studies have demonstrated that probiotics organisms can survive in the gastric transit where the cells as exposed to acidic environment.[5,25]

Probiotics display enormously variable resistance to acid and bile salts and this feature of probiotics is each species and strain-dependent. Procedure to determine the bile resistance: first, bacterial cells are suspended in Man, Rogosa and Sharpe (MRS) broth (originally developed in 1960 by de MRS) with 0.2% and 0.4% of bile salts. Then the broth will pour into three tubes. One as a control incubate in MRS broth without bile salts and other tubes contains 0.2% and 0.4% bile salts, respectively kept for incubation. Finally, look for their optical density by spectrophotometer at 540 nm.[6,25]

Adherence to Intestinal Epithelial Cells

The adherence of probiotic to intestinal mucus and epithelial

cells to colonizes intestinal epithelium have long been considering as one of the most important selection criteria for probiotic microorganisms. Adhesion to the intestinal mucosa may additionally prevent the probiotic cells being washed out and consequently, enabling temporary colonization, immune modulation and competitive exclusions of pathogens. The probiotic strain must adhere to the intestinal wall, colonize and multiply in order to produce enzymes, lactic acids, vitamins, and natural antibiotics.[2,4] During intestinal infections, the adhesion of pathogenic bacteria to mucosal surfaces and disruption of the intestinal microbiota is anticipated. Accordingly, the probiotic bacteria might play protective as well as defensive roles through adhesion and colonization of the mucosal surfaces, effectively competing with pathogens for binding sites and nutrients and immune stimulation.[26,27]

Antimicrobial Activity of Probiotics

The probiotic strain should be capable of producing antimicrobial substances is most important in developing the probiotic supplement and probiotic-rich foods. When administered in adequate amounts, probiotics confer health benefits to the host.[24,28] Probiotics might act antimicrobial activity against pathogens through a variety of mechanisms, including the production of antimicrobial substances, competition with pathogens for nutrients and adhesion sites and stimulation of the immune system. Lactic acid bacteria produce several metabolic compounds such as organic acids, fatty acids, hydrogen peroxide, and diacetyl that have antimicrobial activity. Yet, bacteriocins or proteinaceous substances with specific inhibitory activity against closely related species are most studied.[29,30]

MECHANISMS OF PROBIOTICS ACTION

Enhancement of Epithelial Barrier Function

Probiotics are able to influence many of the components of epithelial barrier function by decreasing apoptosis of intestinal cells. *Lactobacillus rhamnosus* GG was able to prevent cytokine-induced apoptosis in intestinal epithelial cell models by inhibiting tumor necrosis factor (TNF).[31] Integral to the gut barrier defense is mucus which is composed of mucins, which are secreted from the goblet cells. Mucin polymerization provides the structural foundation of the mucus, granting protection from pathogens, enzymes, toxins, dehydration, and abrasion. Some of the probiotics like lactobacilli, for instance, have been shown to modulate the regulation of several genes encoding adherence junction proteins such as E-cadherin and β-catenin in T84 epithelial cells.[32,33]

Competition for Adherence

Probiotic competition for adhesion sites on the intestinal epithelium can prevent the formation of colonies of pathogenic bacteria. Probiotic microorganisms compete with invading pathogens for binding sites to epithelial cells and the overlying mucus layer in a strain-unique manner. Once the probiotic adheres to the cell, different biological activities take place, which primarily include the release of cytokines and chemokines. Then, they exert their secondary activity such as stimulation of mucosal and systemic host immunity. For instance, *Saccharomyces boulardii*, a non-lactic acid bacterium, secretes a heat-labile factor that has shown to be responsible for the decreased bacterial adherence.[34-36]

Competitive Exclusion of Pathogenic Microorganisms

Probiotic bacteria are able to exclude or reduce the growth of pathogens by colonization of favorable sites of adhesion such as the intestinal villus and colonic crypts, or excretion of the mucins (MUC2 and MUC3) from goblet cells which inhibits the adherence of enteropathogenic bacteria. Lactic acid bacteria produce several metabolic compounds such as acetic acid and lactic acid that induces a hostile microenvironment by the reducing of the pH of the gut below than what's critical for the survival of pathogenic bacteria. In addition, Wang et al[37] showed that lactic acid could even completely inhibit growth of pathogens inclusive of *E. coli*, *Salmonella* and *L. monocytogenes*. The others include physical blocking of available bacterial receptor sites[32,38,39]; compete with pathogenic bacteria for essential nutrients and energy source; secretion of antimicrobial substances and release of selective gut protective metabolites like arginine, glutamine, short-chain fatty acids and conjugated linoleic acids.[35,40]

Production of Antimicrobial Substances

Probiotics have been shown to suppress pathogen growth through the release of a variety of antimicrobial factors like defensins, bacteriocins and short-chain fatty acids, such as lactic and acetic acids, which reduce the pH of the lumen. Short-chain fatty acids can disrupt the outer membranes of gram-negative pathogens causing inhibition of pathogen growth.[41-43] Bacteriocins are antimicrobial compounds produced by gram-positive bacteria usually the lactic acid bacteria include lactacin B from *Lactobacillus acidophilus*, plantaricin from *L. plantarum* and nisin from *Lactococcus lactis*. These have a narrow activity spectrum and act only against closely related bacteria, but some bacteriocins are also active against food-borne pathogens. The common mechanisms of bacteriocin-mediated killing include the destruction of target cells by pore formation and/or inhibition of cell wall synthesis.[39,40]

Modulation of the Immune System

Probiotics have the capability to enhance the immune system by increasing the phagocytic capacity of macrophages, enhancing natural killer cell activity, stimulating immunoglobulin A (IgA) production, and modulation of cytokine production.[44,45]

Interference with Quorum Sensing Signaling Molecules

Quorum sensing or auto-inducers are chemical signaling molecules used for bacterial communication with each other as well as with their surrounding environment. This phenomenon of communication is one of its characteristics that control the gene expression. Probiotic bacteria such as lactobacillus, bifidobacterium and *Bacil-*

lus cereus strains degrade the auto-inducers of pathogenic bacteria by enzymatic secretion or production of auto-inducer antagonists and thereby control the virulence gene expression in pathogenic bacteria.[32,38]

APPLICATION OF PROBIOTICS IN POULTRY PRODUCTION

Probiotics have been reported to increase feed efficiency and productivity of laying hens with an improvement in egg quality by decreased yolk cholesterol level, improved shell thickness and egg weight. Similarly, probiotics such as Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida and Saccharomyces have shown beneficial effect on broiler performance species with evidence of increased resistance of chickens to *Salmonella*, *Escherichia coli* or *Clostridium perfringens* infections.[9,46]

Role against Pathogens Infection

Probiotics have a great role in the stimulation of protective immune response and help to suppress the growth of potential gut pathogens in poultry. The inhibition of pathogen by probiotics is suggested to occur *via* competition for adherence sites on the intestinal wall and nutrient as well as the production of antimicrobial compounds.[10,47] Probiotics such as lactic acid bacteria have been widely known for its importance in exerting inhibitory and antagonistic effects against pathogenic bacteria. Numerous studies have been reported that probiotics can exert antimicrobial effect against pathogenic bacteria *via* the production of metabolites.[2,43,48]

Intestinal colonization with probiotic Lactobacillus strains has been demonstrated to have a preventive function against *Salmonella enterica* serovar enteritidis infection in chicken.[33,49] On the other hand, bacteriocins with antimicrobial properties have been reported to show promising growth inhibition potential against intestinal pathogenic bacteria. Bacteriocins derived from *Lactobacillus salivarius* exhibit strong antagonistic activity against *Campylobacter jejuni* and Gram-positive bacteria Pilasombut et al,[50] reported that oral inoculation of *Bacillus subtilis* spores could reduce intestinal colonization of *E. coli* O78: K80 in chickens.[50]

Role on stimulation of Immune Responses

According to Kabir et al,[7] the dynamics of probiotics related to immune responses demonstrated that antibody production was elevated in broilers after fed with probiotics containing Lactobacillus. The modulation of immune responses by probiotics is also apparently observed in broilers exposed to stress conditions. Lactobacillus-based probiotics administration was observed to improve heat-stress related problems in broilers which are accompanied by improved antibody production as compared to controls. Supplementation of probiotic Lactobacillus in broilers' diet revealed that probiotic could enhance intestinal immunity against coccidiosis by altering the population of intestinal intraepithelial lymphocyte expressing surface markers cluster of differentiation 4 (CD4).[51,52]

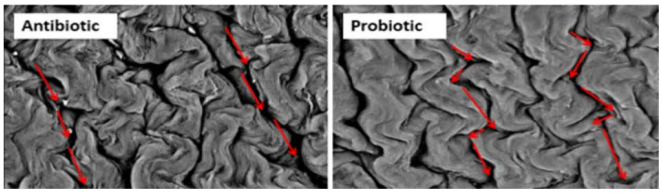
Probiotics have also been suggested to augment Toll-like receptor signaling in which Toll-like receptor plays a crucial role in the activation of T-cells in the intestinal immune system. A recent study showed that probiotic products consists of *Lactobacillus fermentum* and *Saccharomyces cerevisiae* increased the level of messenger ribonucleic acid (mRNA) expression of Toll-like receptors-2 (TLR-2) and 4 in the foregut of the chickens compared to those administered with control diet and antibiotic.[48] Furthermore, basal diet supplemented with probiotics mixture containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobacterium thermophilus* elevated the concentration of IgG and IgM levels in turkeys and the enhancement of the immunoglobulins level have been proposed to contribute to more positive growth performance, production and resistance to diseases.[53]

Effects on Intestinal Morphology

Several studies have been carried out to assess the effects of probiotic administration on the histomorphology of the intestine. According to these studies, dietary treatment with probiotic Lactobacillus species such as *Lactobacillus sakei* Probio-65 was reported to influence the villi height and crypt depth in the small intestine especially jejunum of broilers. Probiotics are proposed to increase the length of villi by activating cell mitosis and induce gut epithelial-cell proliferation.[39,48,54] Increased villi height by probiotics is beneficial to the broilers as the increased surface area of the villi enhanced the absorption of nutrients. It has been suggested that alteration in villi length and crypt depth may lead to poor nutrient absorption, digestive enzymes secretion in the GI tract and eventually lower growth performance in broilers.[8,55,56]

Pelicano et al[57] has described that villi in jejunum occur in zig-zag form, resembling wave pattern. It was suggested that the formation of villi in the wave pattern enables better nutrient absorption than villi arranged in parallel or randomly positioned. Zigzag flux in the small intestine permits food to take a longer passage through the alimentary canal compared to the straight flux, and improve the contact between the nutrients and the absorption surface of the intestinal epithelium. Probiotic such as *Lactobacillus sakei* Probio-65 promoted waved-like arrangement of jejunum villi in broilers (Figure 1).

Figure 1. *Scanning Electron Microscopy of Jejunal Villi Arrangement in Broiler Chickens Administered with Antibiotics and Probiotics*

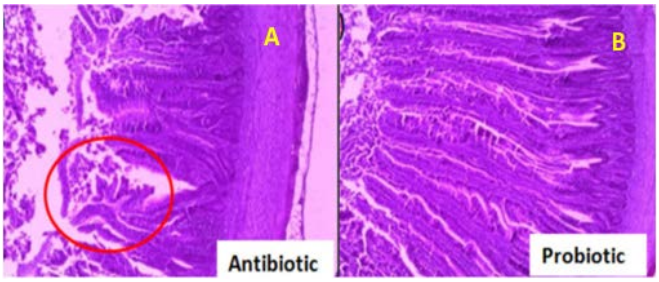


Source: Pelicano et al[57]

Accordingly, the promotion of gut health by probiotic bacteria further strengthens the potential of probiotics as emerging alternatives to antibiotics as growth promoters in poultry production. Gut condition was well preserved in the presence of

probiotics such as *Lactobacillus sakei* Probio-65, accompanied by healthy development of the intestines of as compared to control broilers that were not fed with probiotics. In contrast to probiotics, antibiotic damaged jejunal villi tip with prevalent shedding at the end of the villi tips (red circle in Figure 2). Injuries of the intestinal walls have been much reported upon the administration of antibiotics, and are very often accompanied by thinning of the intestinal mucus layer and increased depletion of goblet cells.[58]

Figure 2. *Histological Images of Sections from Jejunum Tissue of Broiler Chickens Fed with (A) Antibiotic bacitracin (B) Probiotic Lactobacillus Sakei Probio-65*



Source:Wlodarska et al[58]

Role in Growth Performance

The role of probiotics as dietary supplementation and growth performance has been extensively investigated in poultry production. Most studies indicated that probiotics shown great efficacy in promoting animal growth. Lactobacillus inclusion in broilers nutrition also resulted in a higher broiler productivity index, which is measured based on daily weight gain, feed efficiency, and mortality. While growth rates of the broilers are improved, the Lactobacillus administration reduced the mortality of the broilers which usually arose from pathogen infections. Moreover, probiotics supplementation to diet improved feed intake, feed efficiency, and carcass yield of broilers.[3,59,60]

According to recent investigations on the effects of probiotic supplementation on digestive enzymes activity in broiler chickens revealed that the probiotic *Bacillus coagulans* NJ0516 promotes higher activity of protease and amylase. This finding suggests that the higher activity of the enzymes may lead to better digestibility of protein and starch, which in turn explains better growth in broilers fed with probiotics rather than control basal diet.[27] On the other hand, dietary supplementation of probiotic *Lactobacillus sporogenes* lowered serum level of total cholesterol, low-density lipoprotein (LDL) cholesterol, very-low-density lipoprotein (VLDL) cholesterol and triglycerides.[2,16,47]

Role on Quality Poultry Products

Probiotics increase egg production, improve egg quality and decrease egg contamination. Further, probiotics increase eggshell weight, shell thickness and serum calcium in layers and also diets supplemented with commercial probiotic improves and decreased broken egg ratio in layers. According to Panda et al,[47] dietary preparation of *Lactobacillus sporogenes* at 100 mg (6×10^8 spores) per kg of diet significantly increased egg production, eggshell strength, shell weight and shell thickness in laying hens without affecting egg weight, specific gravity, and Haugh unit.[3,9,61]

Probiotics supplementation improves the meat quality in broilers which is recognized all over the world. Intramuscular lipid content is involved in determining meat quality particularly nutrition, tenderness, odor, tastes and flavor characteristics. Greater tendency of higher ratio of unsaturated fatty acids to saturated fatty acids in pectoral and thigh meat of broilers fed with probiotics-supplemented diet. The results suggested that the fat in meat was converted into favorable fat in the presence of probiotics, which in turn contributed to meat tenderness. In broilers, improved tenderness was indicated after mixing their diet with probiotic *Clostridium butyricum*. In contrast to traditional basal diet, the overall organoleptic scores in terms of appearance, texture, juiciness and overall acceptability were higher in probiotic Lactobacillus fed broilers.[62,63]

Meat in broilers fed with probiotics *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium* and Torulopsis species displayed higher content of moisture, protein, and ash compared to the control.[2,64] The results indicated that chicken fed with probiotics has better retention of minerals especially phosphorus, calcium, and nitrogen as well as protein efficiency ratio. According to Hossain et al,[65] a higher protein efficiency ratio may subsequently help promote meat yield. Besides, the addition of probiotics increased breast meat absolute and relative weight. Furthermore, the carcass quality of broilers was also reported to be improved by probiotics with lesser occurrence of Salmonella contamination.[2,7,9]

CONCLUSION

Nowadays, antibiotic resistance and the increase in diseases have posed a great problem in poultry production. Hence, these days the poultry manufacturers' and owners' trend is turning towards natural products. Hereafter, probiotics have come under the scanner for its uses as nutritional supplements. Probiotics are a possible device for lowering intestinal infection by disease-causing and foodborne microorganism. Their benefits to human and animal health have been proven in a lot of Scientific Articles. The use of Probiotics in day-to-day medicine in the treatment of gastrointestinal disorders is increasing with the discovery of the beneficial effect of these agents. Lactobacillus and Bifidobacterium are the main probiotic groups; besides, Pediococcus, Bacillus and yeasts are also another probiotic potential. There are several reports on the role of probiotics as a powerful growth promoter, immune modulator, anti-diarrheal effects, increase product quality and other important properties. Inconclusion, the commercial use of probiotics in poultry production has proceeded because essentially no risk is associated with the consumption of well-defined probiotics in foods and many benefits are possible.

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Review

Review on Epidemiology and Economic Impact of Small Ruminant Brucellosis in Ethiopian Perspective

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ABSTRACT

Brucella are intracellular bacteria that cause brucellosis, a chronic zoonotic disease. The genus of Brucella are subdivided into six species categorized by antigenic variation and primary preferred host and these include *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae*. The epidemiologies of the disease in livestock and humans, as well as appropriate preventive measures, are not well understood in developing countries. Materials excreted from the female genital tract acting as the main supply of organisms for transmission to other animals and human. Millions of individuals are at risk worldwide, especially in countries where infection in animals has not been brought under control, procedures for the heat treatment of milk, such as pasteurization are not routinely applied, and standards of hygiene in animal husbandry are low. A precise diagnosis of brucellosis is important for the control of the disease in animals and consequently in human. Molecular and serological techniques are important tools for diagnosis and epidemiologic studies, providing relevant information for identification of species and biotyping. The economic and public health impact of bucellosis remains particular concern and neglected in developing countries. The disease mainly hampers the productivity of small ruminant's resulting infertility and increase the average inter-calving periods. One of the major gaps in our knowledge at present is the relative contribution of brucellosis on small ruminant and humans. In Ethiopia, no strategy is in place to control brucellosis. The most important approach to the control and prevent human of brucellosis in human and animal is the practice of one health approach. So knowing the status of small ruminant brucellosis in our country is therefore extremely important. Therefore, the aims of this review are; a) To highlight (snapshot) of brucellosis in the small ruminant; b) To show the seroprevalence status of small ruminant brucellosis in Ethiopia perspective; c)To highlight possible risk factor and its economic importance.

Keywords
Small ruminants; Brucellosis; Sheeps; Goats, Ethiopia.

Abbereviations
ASS: Agricultural Sample Survey; CFSPH: Center for Food Security and Public Health; CFT: Complement Fixation Test; CSA: Central Statistical Agency of Ethiopia; ELISA: Enzyme Linked Immuno-Sorbent Assay; FAO: Food and Agricultural Organization; IBM: Interim Brucellosis Manual; Ig: Immunoglobulin; ILCA: International Livestock Center for Africa; MoARD: Ministry of Agriculture and Rural Development; MZN: Modified Ziehl-Neelsen; NVI: National Veterinary Institute; OIE: Office International de Epizootics; RBPT: Rose Bengal Plate Test; WHO: World Health Organization; I-ELISA: Indirect Enzyme Linked Immunosorbent Assay.

INTRODUCTION

Livestock plays a crucial role in the livelihoods of the majority of Africans. It accounts for 16% of the national and 27-30% of the agricultural gross domestic products (GDPs) and 13% of the country's export earnings. The greatest share of this income is from small ruminants.[1] According to the animal population survey results conducted at rural sedentary areas at country level in 2016/17, the estimates livestock of cattle to be about 59.5 million, about 30.70 million sheep, about 30.20 million goats, about 2.16 million horses, about 8.44 million donkeys, about 0.41 million mules, and about 1.21 million camels and poultry population[2]

about 56.53 in Ethiopia. According to statistics from the Central Statistical Agency (CSA),[3] 25% of the sheep and 73% of the national goat population inhabit in the lowlands.

Small ruminants are among important domestic animals which are highly adaptable to a broad range of environmental conditions[4] and they fulfill a number of economic and social functions. Unlike a large number of small ruminant's populations, the country fails to optimally utilize these resources mainly various factors in which diseases stand front line. One of the diseases that hamper the productivity of small ruminant is bucellosis.[5]

The economic and public health impact of bucellosis remains of particular concern in developing countries.[6] The disease can affect almost all domestic species and cross transmission can occur between cattle, sheep, goat, camel and other species.[7,8] Several closely related species of the genus Brucella have been recognized, namely *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, *B. pinnipedialis*, *B.ceti*, *B. microti*, and *B. inopinata*[9] and small ruminants infected by ingestion of contaminated feed or water and the consequences of the infection are determined by the virulence of the bacteria, resistance and reproductive status of the host.[10] In animals, abortion is typically one of the clinical signs of pregnant females, and orchitis and epididymitis are typical clinical signs of the male. Excretion of the organisms in uterine discharges and in milk is common.[11]

Brucellosis is considered as neglected zoonotic disease by the World Health Organization (WHO) and has been identified as having the highest public health burden across all sections of the community. Millions of individuals are at risk worldwide, especially in countries where infection in animals has not been brought under control and standards of hygiene in animal husbandry are low.[12] Materials excreted from the female genital tract are the main supply of organisms for transmission to other animals and man.[13] The disease is transmitted to man mainly by direct contact with infected livestock or through consumption of raw or uncooked animal products.[14] *B. melitensis* (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis and it is highly pathogenic for humans causing udulant or Malta fever followed by *B. suis, B. abortus* and *B. canis* in human.[15]

The epidemiology of the brucellosis livestock and humans as well as appropriate preventive measures is not well understood, and in particular, such information is inadequate in developing countries of Sub-Sahara, including Ethiopia.[16,17] The disease spreads from one herd to another and from one area to another is always due to the movement of infected animals. Hence, lack of biosecurity measures such as strict movement control of animal from one area to another, lack of proper hygienic practices and good husbandry management play a great role in the increment of the prevalence of brucellosis.[18,19]

The control and prevention of brucellosis in farm animals depend on animal species involved, *B. Spp.* management practices and availability and efficacy of vaccines. The options to control the disease include immunization, testing and removal, and improving management practices and movement control.[20] A very important approach to the control of brucellosis that is gaining more and more recognition around the world in recent years is the one health approach to control and prevent human and animal brucellosis.[7]

LITERATURE REVIEW

Description of the Agent

Definition and etiology: Brucellosis in small ruminants is mainly caused by *B. melitensis* and *B. ovis* and in sporadic cases *B. abortus*. *B. melitensis* is most commonly infects sheep and goats. Breed susceptibility is variable in sheep, but goat breeds are highly susceptible. *B. ovis* primarily affects rams[21] and important cause of orchitis and epididymitis in rams and occasionally infects ewes.[22]

Brucellosis is a contagious bacterial disease of an animal which has zoonotic importance, causing significant reproductive losses in animals.[23-25] The genus of Brucella are subdivided into six species categorized by antigenic variation and primary preferred host and these include *B. abortus, B. melitensis, B. suis, B. ovis, B. canis* and *B. neotomae*.[26]

Brucellae are intracellular, coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. The organisms are gram-negative facultative intracellular parasites.[23] Carbon dioxide is important elements for the growth of Brucella organism, especially *B. abortus*; such organisms, which require carbon dioxide for their growth, are called capnophilic organisms. At pH<4, Brucella agents do not have the potential to survive.[27]

Epidemiology

Geographical distribution: Brucellosis is a highly worldwide contagious bacterial disease affecting both animal and human.[28] Remains endemic among Mediterranean countries of Europe, Northern and Eastern Africa, near East countries, India, Central Asia, Mexico and Central and South America.[29] *B. melitensis* is considered to be a re-emerging pathogen in the Middle East[30]; where there is an increasing incidence of *B. melitensis* or *B. suis* biovar 1 infection in cattle.[28]

Among the members of the Brucella group *B. abortus*, *B. melitensis* and *B. suis* species are not host-specific, and may transmit to other animal species; hence, from epidemiological evidence, the three species (*B. abortus, B. melitensis*, and *B. suis*) have distinct host preferences and the organisms are capable to cause an infection in a wide range of host species, including humans. The remaining three members of the species have much greater host specificity. Cross transmission of brucellosis can occur among cattle, swine, sheep and goats and other species including dogs, horses, feral swine, bison, reindeer and camels.[29]

The lack of vaccines for humans will continue to make this disease a global health threat. The lack of a human brucellosis vaccine remains challenging due to the risk of Brucella as a pos-

sible bioweapon agent, and because brucellosis remains a global health problem affecting at least a half million people annually.[31] Even for the most common if the most prevalent *brucella Spp.* of human is *B. melitensis* there is no vaccine provided so far.[32-34]

Source of infection and mode of transmission: Materials excreted from the female genital tract forming the main supply of organisms for transmission to other animals and man. Therefore, in most circumstances, the primary route of dissemination of Brucella is the placenta, fetal fluids and vaginal discharges expelled by infected ewes after abortion or full-term parturition. Very large numbers of organisms are shed at the time of parturition or abortion.[35]

Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, *via* conjunctiva, inhalation and udder contamination during milking or by licking the discharge of an animal, newborn calf or retained fetal membrane.[36] Venereal infections can also occur and mainly infected with *B. suis* infections. The importance of venereal transmission varies with the species; it is the primary route of transmission for *B. ovis, B. suis* and *B. canis* frequently by this route. *B. abortus* and *B. melitensis* can be found in semen, but the venereal transmission of these organisms is uncommon.[10]

B. melitensis frequently occurs in sheep and goats and is highly pathogenic for humans, causing as it does one of the most serious zoonoses in the world.[29,35] The disease is responsible for considerable economic losses to the small-ruminant industry.[13]

In human, brucellosis is spread through contact with blood, body tissues, or body fluids of infected animals. The most common method is the consumption of unpasteurized milk and dairy products. Human infections may occur through breaks in the skin when handling infected animal tissues.[31] In the laboratory and probably in abattoirs, Brucella can be transmitted through aerosols; contact with laboratory cultures and tissue samples; and accidental injection of live Brucella vaccines.[37]

Possible risk factors: Agent factors: Brucella is intracellular pathogen which is able to survive and replicate within phagocytic cells. It can persist on fetal tissues and soil or vegetation for 21-81-days depending on the month, temperature, and exposure to sunlight. *B. abortus* field strain persisted up to 43-days in oil and vegetation at naturally contaminated bison birth or abortion sites.[5]

The organisms are able to survive within host leukocytes and may utilize both neutrophils and macrophages for protection from humoral and cellular bactericidal mechanism during the period of haematogenous spread. The inability of the leukocytes to effectively kill virulent *B. abortus* at the primary site of infection is a key factor in the dissemination to regional lymph nodes and other sites such as recticuloendothelial system and organs such as the uterus and udder.[36] The congregation of a large number of mixed ruminants at water points facilitates disease spread.[11]

Host factor: Population density (number of animals to land area) is attributed to increased contact between susceptible and infected animals. Health status of the animals may also play a great role in acquiring and spread of the disease infection. Vaccinated and disease free animals are less susceptible than unvaccinated and immune compromised diseased animals.[11] Goats are at higher risk of acquiring Brucella infection than sheep. This may be due to the greater susceptibility of goats to Brucella infection. It could also be partly due to the fact that goats excrete the organism for a long period of time, unlike sheep.[36]

The receptivity of ewes to *B. melitensis* varies according to the breed. Milk producing ewes are more receptive than sheep feed lot sheep.[33] Sexually mature and pregnant animals are more prone to brucellosis than sexually immature animals of either sex.[36,38]

Brucellosis sero-prevalence increased with age and sexual maturity. The antibody titer against *Spp.* appears to be associated with age, as a low prevalence in young stock has been reported than the adults.[39-42] This low prevalence in young animals may be explained on the basis that the animal may harbor the organism without expressing any detectable antibodies until their first parturition or abortion. It may be possible that after entry, the organism localizes itself in the regional lymph nodes and enjoy there without provoking antibody production until the animal is conceived and start secreting erythritol, which stimulates and supports the growth of Brucella organisms.[43-46] This is related to the fact that sex hormones and meso-erythritol (in male testicles and seminal vesicles) and erythritol in female, allantoic fluid stimulate the growth and multiplication of Brucella organisms and tend to increase in concentration with age and sexual maturity.[11,47,48]

Reservoir: Carrier animals facilitate the transmission of brucellosis highly by contaminating the environment and also being the site of multiplication for the Brucella organisms in their body and excreting such agents and again the execrated organisms infect animals and humans then bring hazards on health and economy of the country. The carriers are dogs, cats and wild carnivores, such as foxes and wolves, which may be important as mechanical disseminators of infection by carrying away infected material such as fetuses or fetal membranes enhances the viability of the organisms in the environment, thus increasing the chances of infecting susceptible animals.[29]

Environmental and Climatic Factors

The survival of the organism in the environment plays a great role in the epidemiology of the disease.[17]

Brucella may retain infectivity for several months in water, aborted fetuses and fetal membranes, feces and liquid manure, wool, hay, on buildings, equipment and clothes. Brucella is also able to withstand drying particularly in the presence of extraneous organic material and will remain viable in dust and soil.[21] Temperature, humidity and pH influence the organism's ability to survive in the environment. Brucella is sensitive to direct sunlight, disinfectant and pasteurization.[11]

Management: The spread of the disease from one herd to another and from one area to another is always due to the movement of infected animals from an infected herd into a non-infected susceptible herd. Hence, lack of strict movement control of animal from one area to another, lack of proper hygienic practices and good husbandry management play a great role in the increment of the prevalence of brucellosis.[18]

Occupations at higher risk: People who work with animals or come into contact with infected blood are at higher risk of brucellosis. Examples include: veterinarians, dairy farmers, ranchers, slaughterhouse workers, hunters, microbiologists and farmer[7] and also those handling artificial insemination, abattoir and slaughterhouse personnel working in endemic areas are at risk. Brucellae are considered as potential bioweapons.[5]

Pathogenesis

B. melitensis can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, the gastrointestinal tract and through reproductive tracts. In the alimentary tract the epithelium covering the ileal Peyer's patches are preferred site for entry. In the gastrointestinal tract, the organisms are phagocytosed by lymphoepithelial cells of gut-associated lymphoid tissue, from which they gain access to the sub-mucosa and localized to the reticulo-endothelial system and genital organs.[49]

The initiation of Brucella infection depends on exposure dose, the virulence of the *B. Spp.* and natural resistance of the animal to the organisms.[36] They are taken up in phagosomes, remain viable by suppressing phagosome-lysosome fusion, and inhibit apoptosis of host cells. They multiply in vacuoles within the endoplasmic reticulum and from there spread to various organs, particularly into the cells of the reticuloendothelial system, liver, spleen, skeletal muscle, and urogenital tract where they give rise to granulocytic inflammation with or without necrosis or caseations.[5]

After the Brucella organisms spread through the hematogenous route in females then also reaches the placenta and finally to the fetus. The preferential localization to the reproductive tract of the pregnant animal is due to the presence of the allantoic fluid factors that would stimulate the growth of Brucella. Erythritol (four-carbon alcohol) is considered to be one of the factors, which are elevated in the placenta and fetal fluid from about the fifth month of gestation. An initial localization within erythrophagostic trophoblasts of the placentome adjacent to chorioallantoic membrane results in rupture of the cells and ulceration of the membrane. The damage to placental tissue together with fetal infection and fetal stress inducing maternal hormonal changes may cause abortion.[36]

Clinical Feature of Small Ruminant Brucellosis

Disease in animal: In animals, brucellosis can be latent for several years. In females, it manifests itself as abortion, neonatal weakness, retention of the placenta, endometritis and, rarely, mastitis. In males, orchitis, epididymitis and subsequent infertility. In cattle and other animals, polyarthirits, tendovaginitis, and bursitis have been observed.[5] This disease has no pathognomonic lesions leathery placenta and the changes that can be observed are necrotizing placentitis, palpable testicular alterations, necrotizing orchitis and epididymitis with subsequent granuloma, necrotizing seminal vesiculitis and prostatitis. Some aborted fetuses may have an excess of blood-stained fluids in the body cavities, with enlarged spleen and liver. Others appear normal. Infected fetal membranes show changes affecting part or all of the membrane. The necrotic cotyledons lose their blood-red appearance becoming thickened and dull-grey in color. In the chronic stage of the disease, the epididymis can be increased in size up to four or fivefold.[50]

Disease in human: Brucellosis, a foodborne zoonosis has caused considerable morbidity in humans in many parts of the world with major impacts on young children and elderly people. The two species: *B. melitensis* and *B. suis* have been reported to be more virulent in humans.[30]

Human brucellosis is characterized by a variable incubation period (from several days up to several months), and clinical signs include symptoms of continued, intermittent or irregular fever of variable duration, with headaches, weakness, profuse sweating, chills, depression and weight loss. Localized suppurative infections may also occur. Abortion has also happened during the early trimesters of pregnancy.[29] In the chronic form, it may result in serious complications in which the musculoskeletal, cardiovascular and central nervous systems are affected.[51]

Diagnosis

A precise diagnosis of brucellosis is important for the control of the disease in animals and consequently in human. Clinical diagnosis is based usually on the history of reproductive failures in livestock, but it is a presumptive diagnosis that must be confirmed by laboratory methods.[52]

Both in humans and animals, clinically diagnosing of brucellosis is not easily achieved because of the presence of other diseases which have similar clinical signs. Even if clinical history and information about the patient give some clue in humans case, laboratory tests such as screening tests and confirmatory tests are very important tools for a correct identification of the disease in humans and for the detection and confirmation in animals; this enables to take strategic measures for controlling and prevention of brucellosis both in animals and humans accordingly.[27]

The isolation and identification of Brucella offers a definitive diagnosis of brucellosis. It is useful for epidemiological purposes and to monitor the progress of a vaccination program in animals.[29] The method of diagnosis includes the following:

Direct Diagnosis

Microscopic staining: The disease can be confirmed by demonstration of the bacteria in smears. The smears made from vaginal dis-

charges, placenta, colostrum, fetal stomach fluid or of the aborting cow's lochia, and the abomasum of the aborted fetus using the modified Ziehl-Neelsen (MZN) stain.[6,29] Impression smears may be taken from freshly cut and blotted tissue surfaces, e.g. cotyledons, by firmly pressing the slide surface against the tissue. Allow to air dry and heat fix smears. In MZN-stained smears, the bacteria appear as red intracellular coccobacilli whereas most other bacteria stain blue.[53]

Bacteriological culture: Isolation of the organism is considered the golden standard diagnostic method for brucellosis since it is specific and allows biotyping of the isolate, which is relevant under an epidemiological point of view.[13,54] *Brucella Spp.* is classified as a Biosafety level 3 organism, whose manipulation should be performed in biosafety level-3 laboratories. Importantly, brucellosis is one of the most common accidental laboratory infections, particularly in research laboratories.[55]

All Brucella strains are relatively slow growing, and because the specimens from which isolations best attempted are frequently heavily contaminated, the use of a selective medium, e.g. Farrell's medium is advocated.[10] Incubation normally continues for 72-hours, but a negative diagnosis can only be made after weeklong incubation. Specimens which may be used for *B. abortus* isolation include: fetal stomach fluid, spleen, liver, placenta, lochia, milk (especially colostrum or milk within a week of calving), semen and lymph nodes supramammary (chronic and latent infections) and retropharyngeal (early infections) are preferred, but iliac, prescapular and parotid may be used. If serological reactions are thought to be caused by S19 vaccine strain then it is important to collect prescapular lymph nodes as well. All *B. abortus* isolates should be forwarded to laboratories capable of biotyping.[53]

B. Spp. colonies are elevated, transparent, convex, with intact borders, smooth, and a brilliant surface. The colonies have a honey color under transmitted light. Optimal temperature for culture is 37 °C, but the organism can grow under temperatures ranging from 20 °C to 40 °C, whereas optimal pH ranges from 6.6 to 7.4. Some Brucella spp. requires CO_2 for growth. Typical colonies appears 2 to 30 days of incubation, but a culture can only be considered negative when there are no colonies appears 2 to 3 weeks of incubation.[56]

Molecular techniques: Molecular techniques are important tools for diagnosis and epidemiologic studies, providing relevant information for identification of species and biotypes of *Brucella spp.*, allowing differentiation between virulent and vaccine strains.[57] Molecular detection of *Brucella spp.* can be done directly on clinical samples without previous isolation of the organism. In addition, these techniques can be used to complement results obtained from phenotypic tests.[54]

Molecular technologies like, polymerase chain reaction (PCR) is a new approach and applied in many diagnostic works to overcome limitation and difficulties in bacterial culture and serological assays. PCR shows high sensitivity, specificity and overcame the extraneous intervention of mimicry antibodies from sources other than actual infection.[36,58]

PCR and/its variants, based on the amplification of specific genomic sequences of the genus, species or even biotypes of *Brucella spp.*, are the most broadly used molecular technique for brucellosis diagnosis.[54]

Real-time PCR is more rapid and more sensitive than conventional PCR. It does not require post-amplification handling of PCR products, thereby reducing the risk of laboratory contamination and false positive results. Real-time PCR assays have been recently described in order to test Brucella cells.[59]

Indirect Diagnosis

Detection of antibodies and at a lesser degree the measure of the cell-mediated immunity against relevant Brucella epitopes is the more practical approach.[60]

Serological tests are crucial for laboratory diagnosis of brucellosis since most of the control and eradication programs rely on these methods. Inactivated whole bacteria or purified fractions (i.e., lipopolysaccharide or membrane proteins) are used as antigens for detecting antibodies generated by the host during the infection. Antibodies against smooth Brucella species (e.g., *B. abortus, B. melitensis* and *B. suis*) cross reacts with antigen preparations from *B. abortus*, whereas antibodies against rough *Brucella spp.* (e.g., *B. ovis* and *B. canis*) cross react with antigen preparations from *B. ovis*.[61]

Rose bengal plate test: The rose bengal plate test (RBPT) was used as a screening test for the serum samples collected for the presence of Brucella agglutinins. The test was conducted as per the procedure recommended by OIE and Radostits et al.[54,62] The interpretation of the results was done according to the degree of agglutination.[62] Agglutinations were recorded as 0, +, ++ and +++. A score of 0 indicates the absence of agglutination; + indicates barely visible agglutinations; ++ indicates fine agglutination, and +++ indicates coarse clumping. Those samples with no agglutination (0) were recorded as negative while others were recorded as positive. Positive reactions should be investigated using suitable confirmatory and/or complementary strategies (including the performance of other tests and an epidemiological investigation).[53]

Indirect Enzyme Linked Immunosorbent Assay

Enzyme linked immunosorbent assay (ELISA) has become popular as a standard assay for the diagnosis of brucellosis, serologically. It measures IgG, IgA and IgM antibodies and this allows a better interpretation of the clinical situation. The diagnosis of brucellosis is based on the detection of antibodies against the smooth Lipopolysaccharides (LPS). Detection of IgG antibodies is more sensitive than detection of IgM antibodies for diagnosing cases of brucellosis, but specificity is comparable.[56,63] Enzyme-linked immunosorbent assay (ELISA) is an excellent method for screening large populations for Brucella antibodies and for differentiation between

any acute and chronic phases of the disease.[64]

Complement fixation test: The complement fixation test (CFT) is the most widely used test for the serological confirmation of brucellosis in animals. The CFT is both sensitive and specific, in the hands of experienced users, and is used as a definitive (confirmatory) blood serum test.[53] Due to its high accuracy, complement fixation is used as confirmatory test for *B. abortus, B. melitensis*, and *B. ovis* infections and it is the reference test recommended by the Organisation for Animal Health (OIE) for international transit of animals.[65]

In most cases, the CFT is used on RBPT positive sera, but like the RBPT, it is also affected to a large extent by the misuse of strain 19 vaccine, particularly when recent or repetitive vaccinations have been used in sexually mature heifers and cows. It is almost impossible to prescribe strict cutoff readings that indicate infection particularly when S19 vaccination reactions play a role due to its misuse.[53] However, this method has some disadvantages such as high cost, complexity for execution, and requirement for special equipment and trained laboratory personnel. In addition, the test presents limitations with hemolysed serum samples or serum with anti-complement activity of some sera, and the occurrence of prozone phenomena.[66] Sensitivity of complement fixation ranges from 77.1 to 100% and its specificity from 65 to 100%.[67]

Management Strategies

Treatment, control and prevention: The control and prevention of brucellosis in farm animals depend on animal species involved, *Brucella spp.* management practices and availability and efficacy of vaccines. The options to control the disease include immunization, testing and removal, and improving management practices and movement control.[20]

Small ruminant can get infection from carrier animal's sheep, goat and cattle at pasture and water area. It is the same as those for the control of the disease in populations which are already infected.[33] Brucellosis can be controlled by test and slaughter policy and vaccination in other livestock. However, developing courtiers cannot afford test and slaughter approach.[35]

There is general agreement that the most successful method for the prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev. 1 for sheep and goats and *B. abortus* strain have proven to be superior to all others.[35]

A very important approach to the control of brucellosis that is gaining more and more recognition around the world in recent years is the one health approach to control and prevent human and animal brucellosis requires multidiscipline efforts since neither veterinarian alone nor physician alone couldn't perform all approaches of control. So it requires the participation of other discipline and farmers for effective control especially in developing countries where most people are live closer to animals.[7]

In Ethiopia at regional levels, no strategy is in place to control brucellosis.[68] But everybody has own responsibility to keep his environment, animals and own health care. To lower your risk of getting brucellosis from a natural source: Avoid eating or drinking unpasteurized milk, cheese, or ice cream. Check the label to make sure it says "pasteurized" and do not eat it if you aren't sure, do not handle sick or dead animal bodies. But if you must, then use gloves plus face and eye protection, cook meat thoroughly. It is always a good idea to wash your hands regularly and avoid touching your eyes, nose, and mouth and disinfecting of the area where the animals are aborted.[7]

Economic Significance Small Ruminant Brucellosis

Brucellosis presents a significant impediment to the economic potential of the large population of small ruminants. Since small ruminants and their products are an important export commodity, detaining seropositive animals in quarantine has a negative economic impact.[38,69]

Brucellosis is a major veterinary and human health importance in the economy of affected countries. Among the genus Brucella, *B. melitensis, B. abortus, B. suis* and *B. ovis* which preferentially infect sheep and goats, cattle, pigs and sheep, respectively are the most important from a socioeconomic standpoint. In addition to decreasing productivity in animals, the first three species are the main ones responsible for brucellosis in human beings.[70] Since there is close contact between humans and their livestock, which sometimes share the same housing enclosures, brucellosis is a significant health risk to the entire community. This disease causes acute febrile illness–undulant fever–which may progress to a more chronic form and can also produce serious complications affecting the musculoskeletal, cardiovascular, and central nervous systems (CNS). Brucellosis is a zoonotic bacterial disease caused by *Brucella spp.* and is primarily a disease of animals, whereas humans are accidental hosts.[33]

Expansion of animal industries, the lack of hygienic measures in animal husbandry and poor food handling partly account for brucellosis to remain a public health hazard. International travel and the importation of different dairy products into Brucella free regions contribute to the ever-increasing concern over human brucellosis.[6,29] High risk groups include those exposed through occupation in contexts where animal infection occurs, such as slaughterhouse workers, hunters, farmers and veterinarians.[29]

The common sequel of infertility increases the period between lactations in infected herds, and the average inter-calving period may be prolonged by several months. Costs include production loss associated with infection in animals, preventive program, and in the human disease cost of treatment and absenteeism from work brings many economical impacts.[36]

Status of Small Ruminant Brucellosis in Ethiopian Perspective

In Ethiopia, brucellosis in animals and humans has been reported from different localities of the country, particularly associated with

cattle in different agro-ecology and production systems. These prevalence studies in animals and human were largely confined to serological surveys and commonly targeted bovine brucellosis, occasionally sheep and goats and rarely camels.

The existing report on seropositivity for small ruminant in different agro-ecology of Ethiopia reveals ranges 1.2-17.4% for RBPT, and 0.4-13.7% for CFT. Among few reports on the status of small ruminant brucellosis in Ethiopia, tested sera from 2000 sheep and goats in pastoral regions of Ethiopia and documented 1.9% positive using RBPT and 1.7% positive by i-ELISA. Another cross-sectional study conducted on 1568 serum samples from sheep and goats in the pastoral region of Afar revealed 9.37% positive using RBPT and 4.8% positive by CFT.[49] In Jijiga,[71] total of serum samples of sheep and 191 from goats) screened 291 serum samples and the result revealed 1.72% and 1.37% positivity using RBPT and CFT, respectively.[72] showed that from a total of 500 serum samples tested, 1.2% tested positive for brulcellosis infection by the RBPT and only 0.4% was found positive for CFT.[61] Revealed that from a total of 840 blood samples (409 sheep and 431 goats) collected all sera samples were screened by modified rose bengal test (mRBT) positive reactors (4.6%) were also tested positive in I-IELISA.[73] Showed that from a total of 1000 serum samples of 388 sheep and 612 goats at an export abattoir tested, 3.7% tested positive for brulcellosis infection by the RBPT and 2.7% was found positive for CFT. Central and North East Ethiopia revealed that from a total of 2409 blood samples sheep collected all sera samples screened by mRBT positive reactors 4.9% and tested positive 4.89% in CFT. Kombolcha, north east Ethiopia,[74] a total of serum samples 714 (210 from sheep and 504 from goats) screened samples and the result revealed 2.1% and 0.7% positivity using RBPT and CFT, respectively.[75] Indicated that from a total of 414 serum samples (272 from sheep and 142 from goats) collected from Tellalak district, Afar Region showed 17.7% tested positive for brucellosis infection by the RBPT and gave 13.7% counter positive for CFT. In Borena low land the overall sero-prevalence documented by Teshale et al[72] revealed that 8.5% RBPT and 2.3% C. ELISA.[76] In Mojo and Debreziet export abattoir indicated that 1.99% RBPT and 1.6 CFT (Table 1).

Table 1. *Sero-Prevalence Report of Brucellosis in Small Ruminant in Different Locations*

Location	Prevalence (95%,CI)		Source
	RBPT	CFT	
Export Abattoir	3.4	2.7	73
Kombolcha, north east Ethiopia	2.1	0.7	74
Tellalak district, Afar Region	17.4	13.7	75
Mojo and Debreziet export abattoir	1.99	1.76	76
Jijiga zone, Somali region	1.72	1.37	71
Bahir Dar, North West Ethiopia	1.2	0.4	72
Yabellodistricts of Borena Zone	8.5	8.1(c.ELISA)	72
Afar and Somali pastoral areas of Eastern Ethiopia	1.9	1.7	77
Dire Dawa, E/Ethiopia	9.38	9.11	78
Pastora area of Oromia and somale	8.5	3.6	79
Afar	9.37	4.8	49
Arsi and east shoa	4.6	4.6I-ELISA	61
Central and North East Ethiopia	4.98	4.89	78
Dire Dawa, E/Ethiopia	3.5	2.6	80
Chifra and Ewa districts, Afar	13	12.35	68
Borana pastoral system of Ethiopia	2.0	1.6	81

From the over study conducted on small ruminant brucellosis in Ethiopia higher sero-prevalence both in RBPT and CFT were seen from lowland area of the countries. Higher prevalence rate (13.7%) was found in Afar region where commingling of animals at communal grazing is the common practice. while lower prevalence 0.4% was recorded in and around Bahir Dar, North West Ethiopia.[49]

CONCLUSION AND RECOMMENDATIONS

Brucellosis is worldwide and has a high prevalence in different areas of Ethiopia. Brucellosis affects both animals and humans, has a very high economic and public health impact. Its impact on public health is very well related to the infected animal species from which human transmission occurs. The disease is transmitted from infected animals to human beings through several routes. It is a special hazard to occupational groups. It causes considerable losses in small ruminants as a result of abortion and a reduction in milk yield.

Based on the information mentioned conclusion, the following recommendations were forwarded: There should be a strategy to regulate the control mechanism of brucellosis in small ruminants at the national level. Efforts should be made to develop a new vaccine against brucellosis in sheep and goats based on rough strains which are devoid of the disadvantages of the vaccine. The government, public health officers and veterinarians have to work together to reduce the economic and zoonotic impact of brucellosis.

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